

図3. PI3K阻害剤によるT<sub>3</sub>依存性のAkt/PKB, mTOR, p70<sup>S6K</sup>活性化の阻害

- 皮膚腺維芽細胞を甲状腺ホルモン除去FBSを5%含むDMEM中で2日間培養後、T<sub>3</sub>を添加し30分後に細胞を採取した。PI3K阻害剤であるwortmannin (wort, 2 mM)とLY294002 (LY, 50 mM)は、T<sub>3</sub>添加30分前に加えた。
- GFPを発現するコントロールウイルス(AdGFP)或いはΔp85αをM.O.I 200で1時間感染し、甲状腺ホルモン除去FBSを5%含むDMEM中で2日間培養した後、T<sub>3</sub>を添加し30分後に細胞を採取した。

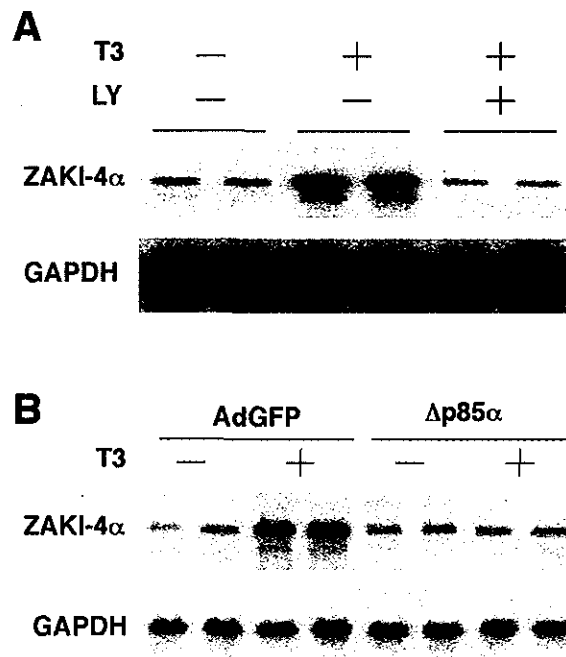


図4. PI3K阻害剤によるT<sub>3</sub>依存性のZAKI-4 $\alpha$ の誘導の阻害

- 皮膚腺維芽細胞を甲状腺ホルモン除去FBSを5%含むDMEM中で2日間培養後、T<sub>3</sub>を添加し12時間後に細胞を採取した。PI3K阻害剤であるLY294002 (LY, 50 mM)は、T<sub>3</sub>添加30分前に加えた。
- GFPを発現するコントロールウィルス(AdGFP)或いは $\Delta$ p85 $\alpha$ をM.O.I 200で1時間感染し、甲状腺ホルモン除去FBSを5%含むDMEM中で2日間培養した後、T<sub>3</sub>を添加し12時間後に細胞を採取した。

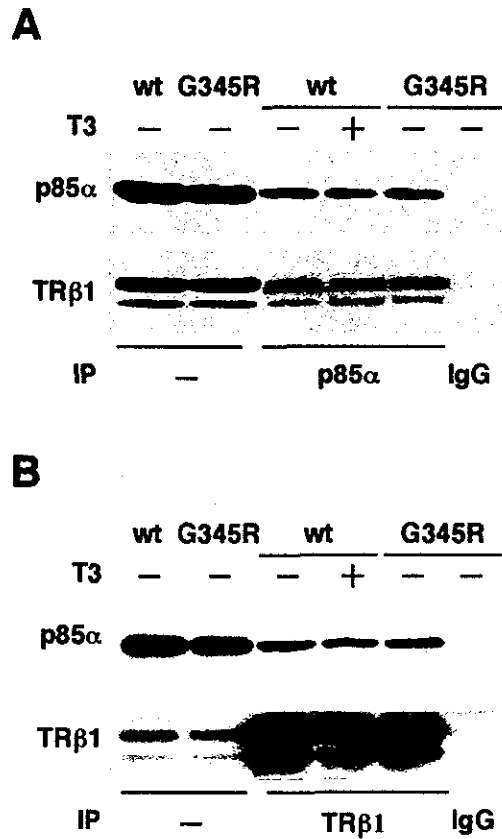


図5. 甲状腺ホルモン受容体 (TR $\beta$ 1) はリガンド非依存性にp85 $\alpha$ と複合体を形成する。

腺維芽細胞を血清を含まないDMEM中で培養し、野生型TR $\beta$ 1 (wt) 或いはドミナントネガティブ作用の強い変異TR $\beta$ 1 (G345R)を発現するアデノウィルスをもO.I 200で1時間感染させ、甲状腺ホルモン除去FBSを5%に含む培養液中で2日間インキュベーションの後、T<sub>3</sub> (10<sup>-8</sup>M)を添加し、30分後に細胞を採取した。全細胞抽出液をAでは抗p85 $\alpha$ 抗体で、Bでは抗TR $\beta$ 1抗体で免疫沈降し、各々の抗体でウェスタンブロットを行った。

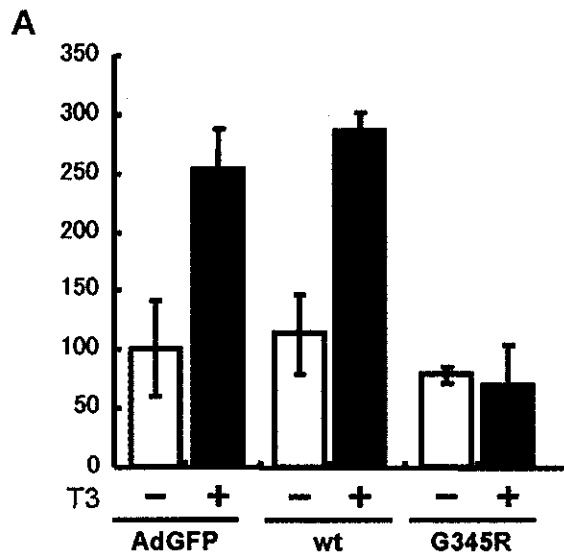


図6. 変異TR $\beta$ 1 (G345)はT<sub>3</sub>依存性のPI3Kの活性化を阻害する

腺維芽細胞を血清を含まないDMEM中で培養し、野生型TR $\beta$ 1(wt)或いはドミナントネガティブ作用の強い変異TR $\beta$ 1 (G345R)を発現するアデノウイルスをM.O.I 200で1時間感染させ、甲状腺ホルモン除去FBSを5%に含む培養液中で2日間インキュベーションの後、T<sub>3</sub> (10<sup>-8</sup>M)を添加し、30分後に細胞を採取した。全細胞抽出液中のPI3Kを抗p85 $\alpha$ 抗体を用いて免疫沈降した。免疫沈降物中のPI3K活性はPI (3,4,5) P<sub>3</sub>の産生をELISAにより測定した。

#### IV. 研究成果の刊行に関する一覧

刊行書籍又は雑誌名 (雑誌のときは雑誌名 巻頁数 論文名)	刊行 年月日	刊行書店名	執筆者氏名
The Journal of Steroid Biochemistry & Molecular Biology 89-90:343-345. Direct action of 1,25-dihydroxyvitamin D on bone: VDRKO bone shows excessive bone formation in normal mineral condition.	2004		<u>Tanaka H, Seino Y.</u>
Caltified Tissue International 75:338-343. Osteoclastogenesis inhibitory factor/ osteoprotegerin reduced bone loss induced by mechanical unloading.	2004		Ichinose Y, <u>Tanaka H</u> , Inoue M, Mochizuki S, Tsuda E, <u>Seino Y.</u>

刊行書籍又は雑誌名 (雑誌のときは雑誌名 巻頁数 論文名)	刊行 年月日	刊行書店名	執筆者氏名
Int J Oncol 24: 89-95 Antitumor effects of peroxisome proliferators activate receptor gamma ligands on anaplastic thyroid carcinoma.	2004		Hayashi N, Nakamori S, Hiraoka N, Tsujie M, Xundi X, Takano T, <u>Amino N</u> , Sakon M, Monden M.
Clin Chim Acta 339: 49-55 Development of radiotransporter assay for determination of iodide: Preliminary experiment.	2004		Miyai K, Minekawa T, Tsutsumi S, Tatsumi K, <u>Amino N</u> .
Clin Chim Acta 340: 187-193 Serum concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate and their relation to cytokine production during and after normal pregnancy.	2004		Tagawa N, Hidaka Y, Takano T, Shimaoka Y, Kobayashi Y, <u>Amino N</u> .
Brit J Cancer 90: 1600-1605 High-throughput differential screening of mRNAs by serial analysis of gene expression: Decreased expression of trefoil factor 3 mRNA in thyroid follicular carcinomas.	2004		Takano T, Miyauchi A, Yoshida H, Kuma K, <u>Amino N</u> .
Eur J Cancer 40: 614-618 PGP9.5 mRNA could contribute to the molecular-based diagnosis of medullary thyroid carcinoma.	2004		Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Nakata Y, Kuma K, <u>Amino N</u> .

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Steroids 69: 675-680 Serum concentration of androstenediol and androstenediol sulfate and their relation to cytokine production during and after normal pregnancy.	2004		Tagawa N, Hidaka Y, Takano T, Shimaoka Y, Kobayashi Y, <u>Amino N.</u>
Clin Endocrinol 61:635-640 A novel <i>PROPI</i> gene mutation (157delA) in Japanese siblings with combined anterior pituitary hormone deficiency.	2004		Tatsumi K, Kikuchi K, Tsumura K, <u>Amino N.</u>
J Clin Endocrinol Metab Nov 9 Detection of monoclonality of the immunoglobulin heavy chain gene in thyroid malignant lymphoma by vectorette PCR.	2004		Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Notomi T, Kuma K, <u>Amino N.</u>
Thyroid Serial changes in thyroid ultrasonogram in a Hashimoto's patient who developed malignant lymphoma.	2004		Matsuzuka F, <u>Amino N</u> , Kuma K, Miyauchi A.
Thyriod. Successful treatment for recurrent painful Hashimoto's thyroiditis by total thyroidectomy.	in press		Ohye H, Fukata S, Kubota S, Sasaki I, Takamura Y, Matsuzuka F, <u>Amino N</u> , Kuma K, Miyauchi A, Kakudo K



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Thyroid Transcutaneous iodine absorption in adult patients with thyroid cancer treated with Povidone-iodine topically at operation.	in press		Tomoda C, Uruno T, Takamura Y, Ito Y, Miya A, Kobayashi K, Matsuzuka F, <u>Amino N</u> , Kuma K, Miyauchi A
World J Suegery Streching exercises to reduce symptoms of postoperative neck discomfort after thyroid surgery: A prospective randomized study.	in press		Takamura Y, Miyauchi A, Tomoda C, Uruno T, Ito Y, Miya A, Kobayashi K, Matsuzuka F, <u>Amino N</u> , Kuma K
Endocr J Reevaluation of stringent low iodine diet for the outpatient preparation for radioiodine examination and therapy.	in press		Tomoda C, Kitano H, Uruno T, Takamura Y, Ito Y, Miya A, Kobayashi K, Matsuzuka F, <u>Amino N</u> , Kuma K, Miyauchi A
Thyroid Fetal cell carcinogenesis: A new hypothesis for better understanding of thyroid carcinoma.	in press		Takano T, <u>Amino N</u> .

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Endocrinology 145(5): 2524-2530 Transgenic Mice Producing MHC Class II Molecules on Thyroid Cells Do Not Develop Apparent Autoimmune Thyroid Diseases	2004	The Endocrine Society	Li YS, Kanamoto N, Hataya Y, Moriyama K, Hiratani H, Nakao K, <u>Akamizu T</u>
Eur J Endocrinol.150: 447-455 Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects	2004	EFES	<u>Akamizu T</u> , Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K and Kangawa K
Endocrinology 145 (9): 4144-4153 Genomic Structure and Characterization of the 5'-Flanking Region of the Human Ghrelin Gene	2004	The Endocrine Society	Kanamoto N, <u>Akamizu T</u> , Tagami T, Hataya Y, Moriyama K, Takaya K, Hosoda H, Kojima M, Kangawa K and Nakao K
Eur J Endocrinol. 150: 913-934 Short-term secretory regulation of active form of ghrelin and total ghrelin during oral glucose tolerance test in patients with anorexia nervosa	2004	EFES	Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, <u>Akamizu T</u> , Kangawa K

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Endocrinology 146 (1): 355-36 Transgenic Mice Overexpressing Des-Acyl Ghrelin Show Small Phenotype.	2005	The Endocrine Society	Ariyasu H, Takaya K, Iwakura H, Hosoda H, <u>Akamizu T</u> , Arai Y, Kangawa K, and Nakao K
The Journal of Clinical Endocrinology & Metabolism 90 (1):6-9 Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay	2005	The Endocrine Society	<u>Akamizu T</u> , Shinomiya T, Irako T, Fukunaga M, Nakai Y, Nakai Y, Kangawa K

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Vitamin D: Second Edition, Volume 1, David Feldman, Glorieux Francis H, Pike Wesley J, 851-861 Vitamin D and Reproductive Organs	2004	Academic Press	<u>Ozono K</u> , Nakajima S, Michigami T
J Biol Chem, 279(16):15897-15907 C-3 epimerization of vitamin D3 metabolites and further metabolism of C-3 epimers: 25-hydroxyvitamin D3 is metabolized to 3-epi-25-hydroxyvitamin D3 and subsequently metabolized through C-1alpha or C-24 hydroxylation.	2004		Kamao M, Tatematsu S, Hatakeyama S, Sakaki T, Sawada N, Inouye K, <u>Ozono K</u> , Kubodera N, Reddy GS, Okano T
J Bone Miner Res,19(7):1154-1164 Role of TAFII-17, a VDR binding protein, in the increased osteoclast formation in Paget's Disease.	2004		Kurihara N, Reddy SV, Araki N, Ishizuka S, <u>Ozono K</u> , Cornish J, Cundy T, Singer FR, Roodman GD
J Cell Biochem, 93(2):418-426 Involvement of phosphoinositide 3-kinase signaling pathway in chondrocytic differentiation of ATDC5 cells: Application of a gene-trap mutagenesis.	2004		Ihara-Watanabe M, Uchihashi T, Miyouchi Y, Sakai N, Yamagata M, <u>Ozono K</u> , Michigami T

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Development, 131(21):5469-5480 Skeletal defects in ringelschwanz mutant mice reveal that Lrp6 is required for proper somitogenesis and osteogenesis.	2004		Kokubu C, Heinzmann U, Kokubu T, Sakai N, Kubota T, Kawai M, Wahl MB, Galceran J, Grosschedl R, <u>Ozono K</u> , Imai K
Clin Pediatr Endocrinol, 14(1):11-16 Longitudinal observation of a patient with Leri-Weill dyschondrosteosis and SHOX Haploinsufficiency.	2004		Miyoshi Y, Miki K, Etani Y, Mushiake S, Shimizu N, <u>Ozono K</u>

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EMBO J., 23, 1598-1608. Transrepression by a liganded nuclear receptor via a bHLH activator through co-regulator switching.	2004	European Molecular Biology Organization	Murayama, A., Kim, M., Yanagisawa, J., Takeyama, K., <u>Kato, S.</u>
Proc. Natl. Acad. Sci. USA, 101, 1673-1678. Brain masculinization requires androgen receptor function.	2004	The National Academy of Sciences of the USA	Sato, T., Matsumoto, T., Kawano, H., Watanabe, T., Uematsu, Y., Sekine, K., Fukuda, T., Aihara, K., Krust, A., Yamada, T., Nakamichi, Y., Yamamoto, Y., Nakamura, T., Yoshimura, K., Yoshizawa, T., Metzger, D., Chambon, P., <u>Kato, S.</u>
J. Biol. Chem., 279, 40255-40258. Wnt/beta -catenin and estrogen signaling converge in vivo.	2004	The American Society for Biochemistry and Molecular Biology	Kouzmenko, A. P., Takeyama, K., Ito, S., Furutani, T., Sawatsubashi, S., Maki, A., Suzuki, E., Kawasaki, Y., Akiyama, T., Tabata, T., <u>Kato, S.</u>

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<p>Biochem. Biophys. Res. Commun., 320, 262-267.</p> <p>Juvenile hormones antagonize ecdysone actions through co-repressor recruitment to EcR/USP heterodimers.</p>	2004	Elsevier Ltd.	Maki, A., Sawatsubashi, S., Ito, S., Shirode, Y., Suzuki, E., Zhao, Y., Yamagata, K., Kouzmenko, A., Takeyama, K. <u>Kato, S.</u>
<p>Biochem. Biophys. Res. Commun., 320, 268-272.</p> <p>Ecdysone receptor-dependent gene regulation mediates histone poly (ADP-ribosyl)ation.</p>	2004	Elsevier Ltd.	Sawatsubashi, S., Maki, A., Ito, S., Shirode, Y., Suzuki, E., Zhao, Y., Yamagata, K., Kouzmenko, A., Takeyama, K., <u>Kato, S.</u>
<p>Biosci. Biotechnol. Biochem., 68, 1209-1215.</p> <p>A novel genetic system for analysis of co-activators for the N-terminal transactivation function domain of the human androgen receptor.</p>	2004	Japan Society for Biochemistry, Biotechnology and Agrochemistry	Takeyama, K., Ito, S., Sawatsubashi, S., Shirode, Y., Yamamoto, A., Suzuki, E., Maki, A., Yamagata, K., Zhao, Y., Kouzmenko, A., Tabata, T., <u>Kato, S.</u>
<p>Oncogene, 23, 6000-6005.</p> <p>BRCA1 function mediates a TRAP/DRIP complex through direct interaction with TRAP220.</p>	2004	Nature Publishing Group	Wada, O., Oishi, H., Takada, I., Yanagisawa, J., Yano, T., <u>Kato, S.</u>

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Genes to Cells, 9, 983-992. In vivo potentiation of human oestrogen receptor $\alpha$ by Cdk7-mediated phosphorylation.	2004	Blackwell Publishing Ltd.	Ito, S., Takeyama, K., Yamamoto, A., Sawatsubashi, S., Shirode, Y., Kouzmenko, A., Tabata, T., <u>Kato, S.</u>
J. Steroid Biochem. & Mol. Biol., 89-90, 173-178. Vitamin D receptor (VDR) promoter targeting through a novel chromatin remodeling complex.	2004	Elsevier Ltd.	<u>Kato, S.</u> , Fujiki, R., Kitagawa, H.
J. Steroid Biochem. & Mol. Biol, 89-90, 627-633. Function of androgen receptor in gene regulations.	2004	Elsevier Ltd.	<u>Kato, S.</u> , Matsumoto, T., Kawano, H., Sato, T., Takeyama, K.
EMBO J., 23, 4813-4823. Ligand-dependent switching of ubiquitin-proteasome pathways for estrogen receptor.	2004	European Molecular Biology Organization	Tateishi, Y., Kawabe, Y., Chiba, T., Murata, S., Ichikawa, K., Murayama, A., Tanaka, K., Baba, T., <u>Kato, S.</u> , Yanagisawa, J.
British J. Dermatology. Vitamin D receptor ablation alters skin architecture and homeostasis of dendritic epidermal T cells.	2004 (in press)	Blackwell Science	Meindl, S., Rot, A., Hoetzenecker, W., <u>Kato, S.</u> , Cross, S., Elbe-Burger, A.



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Mol. Endocrinol., 18, 1131-1143. Protein phosphatase 5 is a negative regulator of estrogen receptor-mediated transcription.	2004	The Endocrine Society	Ikeda, K., Ogawa, S., Tsukui, T., Horie-Inoue, K., Ouchi, Y., <u>Kato, S.</u> , Muramatsu, M., Inoue, S.
Genes to Cells, 9, 143-151. Regulation of transforming growth factor- $\beta$ and bone morphogenetic protein signalling by transcriptional coactivator GCN5.	2004	Blackwell Publishing Ltd.	Kahata, K., Hayashi, M., Asaka, M., Hellman, W., Kitagawa, H., Yanagisawa, J., <u>Kato, S.</u> , Imamura, T., Miyazono, K.
J. Biol. Chem., 279, 35798-35802. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice.	2004	The American Society for Biochemistry and Molecular Biology	Aihara, K. I., Azuma, H., Akaike, M., Ikeda, Y., Yamashita, M., Sudo, T., Hayashi, H., Yamada, Y., Endoh, F., Fujimura, M., Yoshida, T., Yamaguchi, H., Hashizume, S., Kato, M., Yoshimura, K., Yamamoto, Y., <u>Kato, S.</u> , Matsumoto, T.

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J. Bone Miner. Res., 19, 1452-1461. SRC-1 is necessary for skeletal responses to sex hormones in both males and females.	2004	American Society for Bone and Mineral Research	Yamada, T., Kawano, H., Sekine, K., Matsumoto, T., Fukuda, T., Azuma, Y., Itaka, K., Chung, U. I., Chambon, P., Nakamura, K., <u>Kato, S.</u> , Kawaguchi, H.
Eur. J. Neurosci., 19, 2826-2838. Targeted introduction of V642I mutation in amyloid precursor protein gene causes functional abnormality resembling early stage of Alzheimer's disease in aged mice.	2004	Federation of European Neuroscience Societies	Kawasumi, M., Okada, T., Yamada, M., Miyamae-Kaneko, M., Matsuoka, M., Nakahara, J., Tomita, T., Iwatsubo, T., <u>Kato, S.</u> , Aiso, S., Nishimoto, I., Kouyama, K.
Am. J. Physiol. Renal. Physiol., 287, F39-F47. Intestinal Na-Pi cotransporter adaptation to dietary Pi content in vitamin D receptor null mice.	2004	The American Physiological Society	Segawa, H., Kaneko, I., Yamanaka, S., Ito, M., Kuwahata, M., Inoue, Y., <u>Kato, S.</u> , Miyamoto K.

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Mol. Carcinogenesis, 41, 77-84. The United States-Japan workshop on: the role of nuclear receptors in carcinogenesis.	2004	Wiley-Liss, Inc.	Peters, J. M., <u>Kato, S.</u> , Gonzalez, F.
Biochem. Biophys. Res. Commun., 323, 505-511. Purification and characteraization of mouse CYP27B1 overproduced by an Escherichia coli system coexpressing molecular chaperonins GroEL/ES.	2004	Elsevier Ltd.	Uchida, E., Kagawa, N., Sasaki T., Urushino, N., Sawada, N., Kamakura, M., Ohta, M., <u>Kato, S.</u> , Inouye, K.
Mol. Endocrinol., 18, 127-141. Protein kinase A potentiates Ad4BP/SF-1 transactivation by re-integrating the subcellular dynamic interactions of the nuclear receptor with its cofactors, GCN5/TRRAP, and suppressor, DAX-1: a laser confocal imaging study in living KGN cells.	2004	The Endocrine Society	Fan, W., Yanase, T., Wu, Y., Kawate, H., Saitoh, M., Oba, K., Nomura, M., Okabe, T., Goto, K., Yanagisawa, J., <u>Kato, S.</u> , Takayanagi, R., Nawata, H.
Biochem. Biophys. Res. Commun., 327, 933-938. TRRAP as a hepatic coactivator of LXR and FXR function.	2005	Elsevier Ltd.	Unno, A., Takada, I., Takezawa, S., Oishi, H., Baba, A., Shimizu, T., Tokita, A., Yanagisawa, J., <u>Kato, S.</u>

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Carcinogenesis, 26, 429-440. 1 $\alpha$ ,25-Dihydroxyvitamin D3 is a preventive factor in the metastasis of lung cancer.	2005	Oxford University Press	Nakagawa, K., Kawaura, A., <u>Kato, S.</u> , Takeda, E., Okano, T.
Am. J. Physiol. Cell Physiol., 288, C429-C434. Intestinal and renal adaptation to a low Pi-diet of type II Na-Pi-cotransporters in VDR and 1 $\alpha$ -OHase deficient mice.	2005	The American Physiological Society	Capuano, P., Radanovic, T., Wagner, C. A., Bacic, D., <u>Kato, S.</u> , Uchiyama, Y., St-Arnaud, R., Murer, H., Biber, J.