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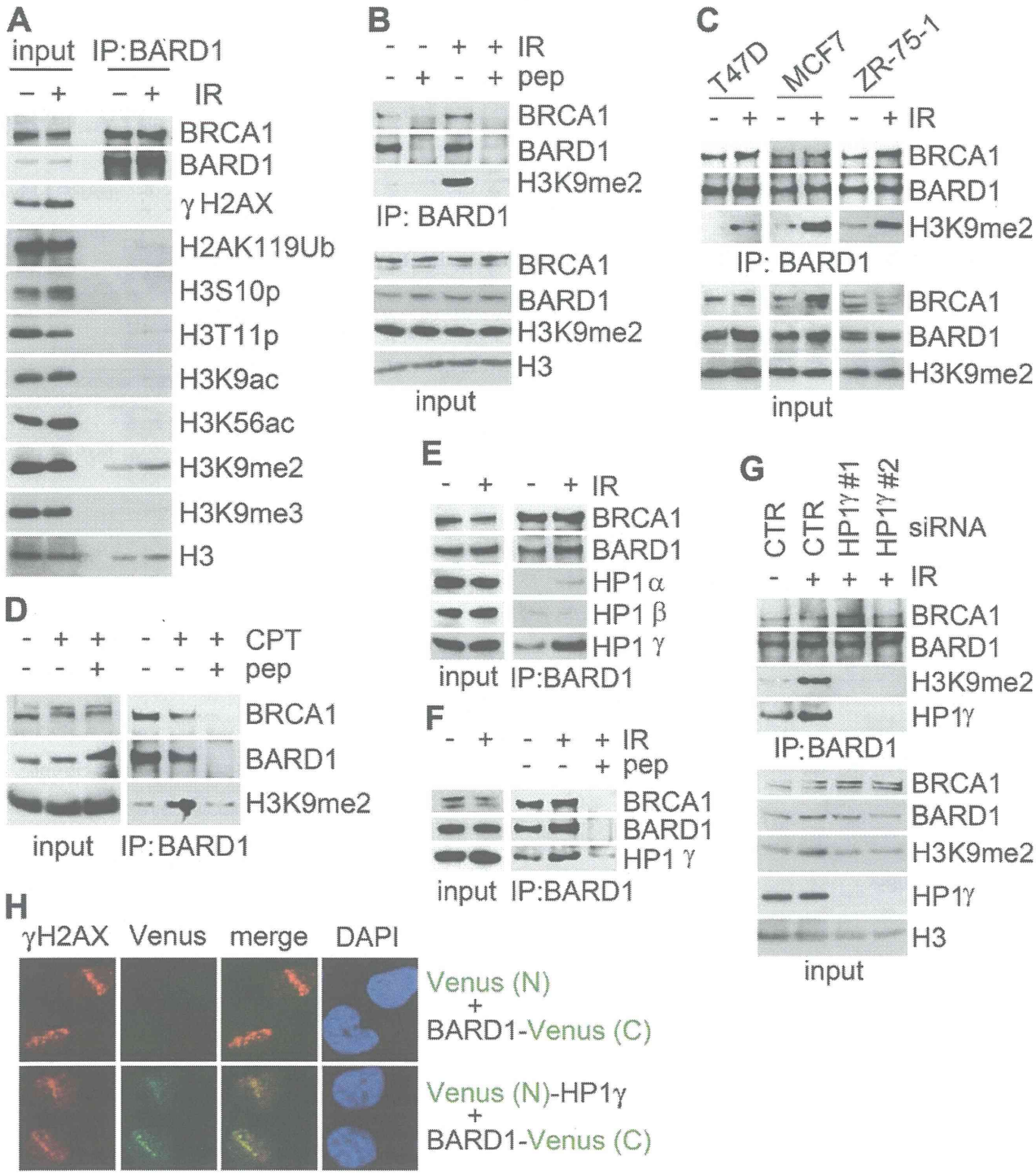
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Wu_Fig. 1



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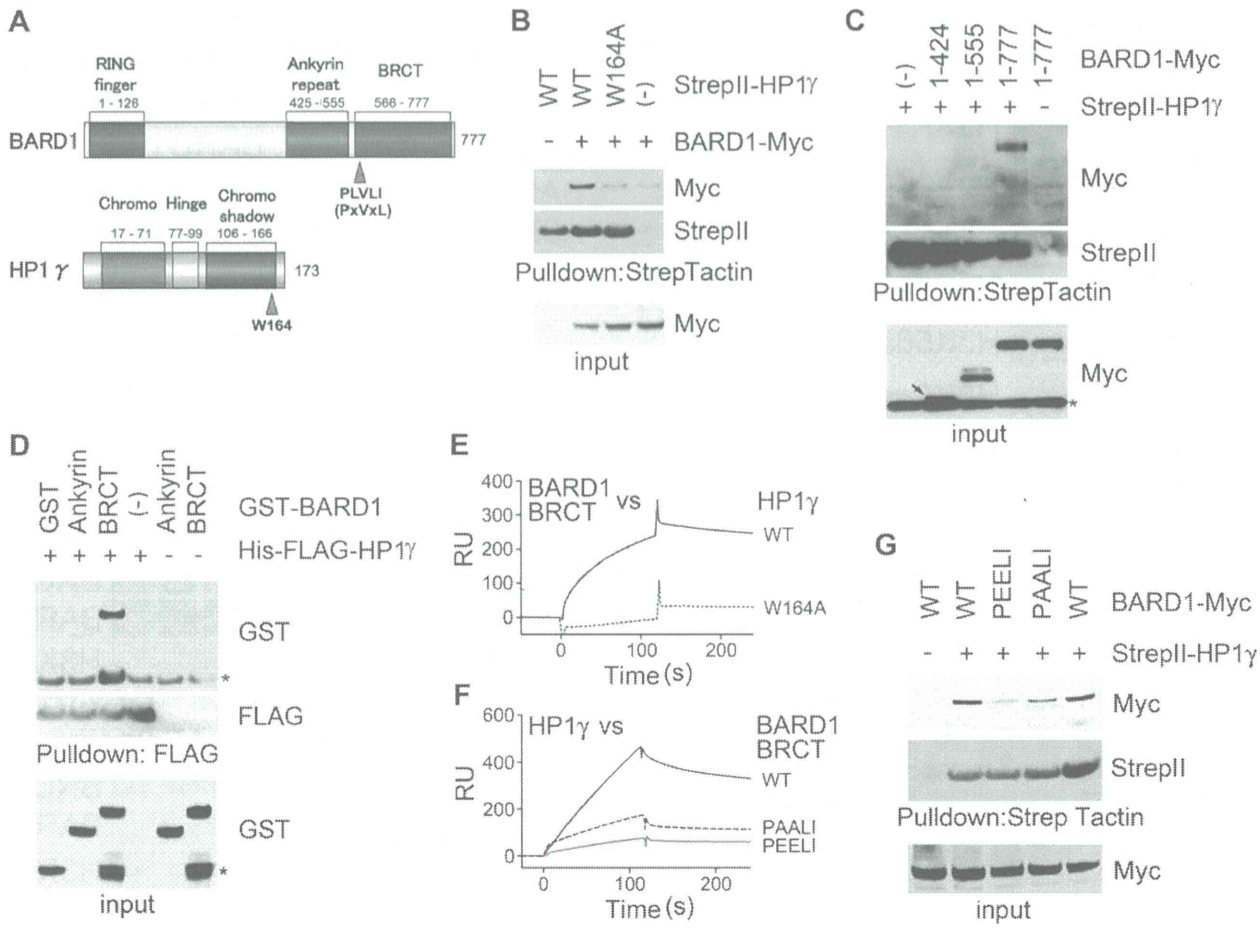
Wu_Fig. 2



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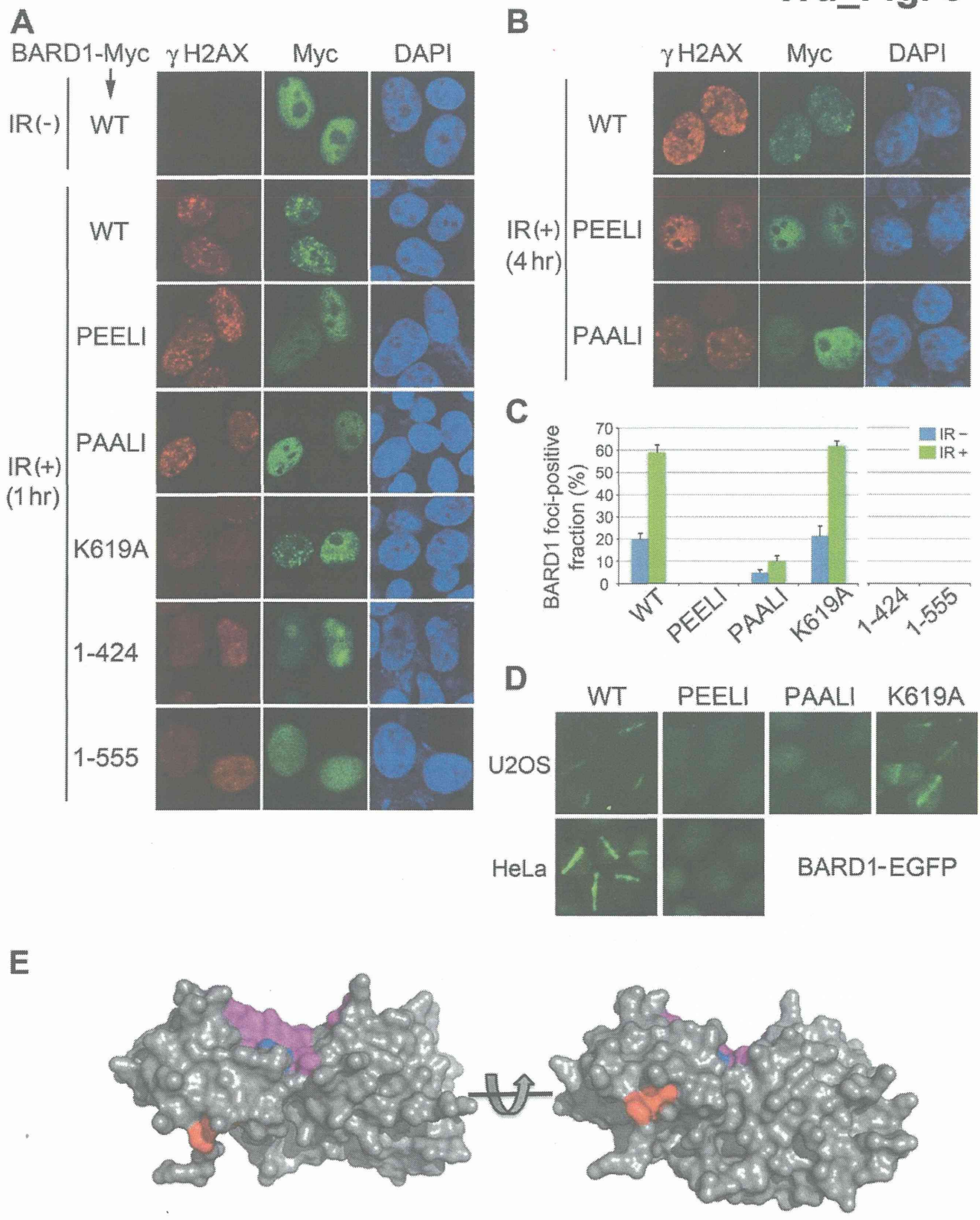
Wu_Fig. 3



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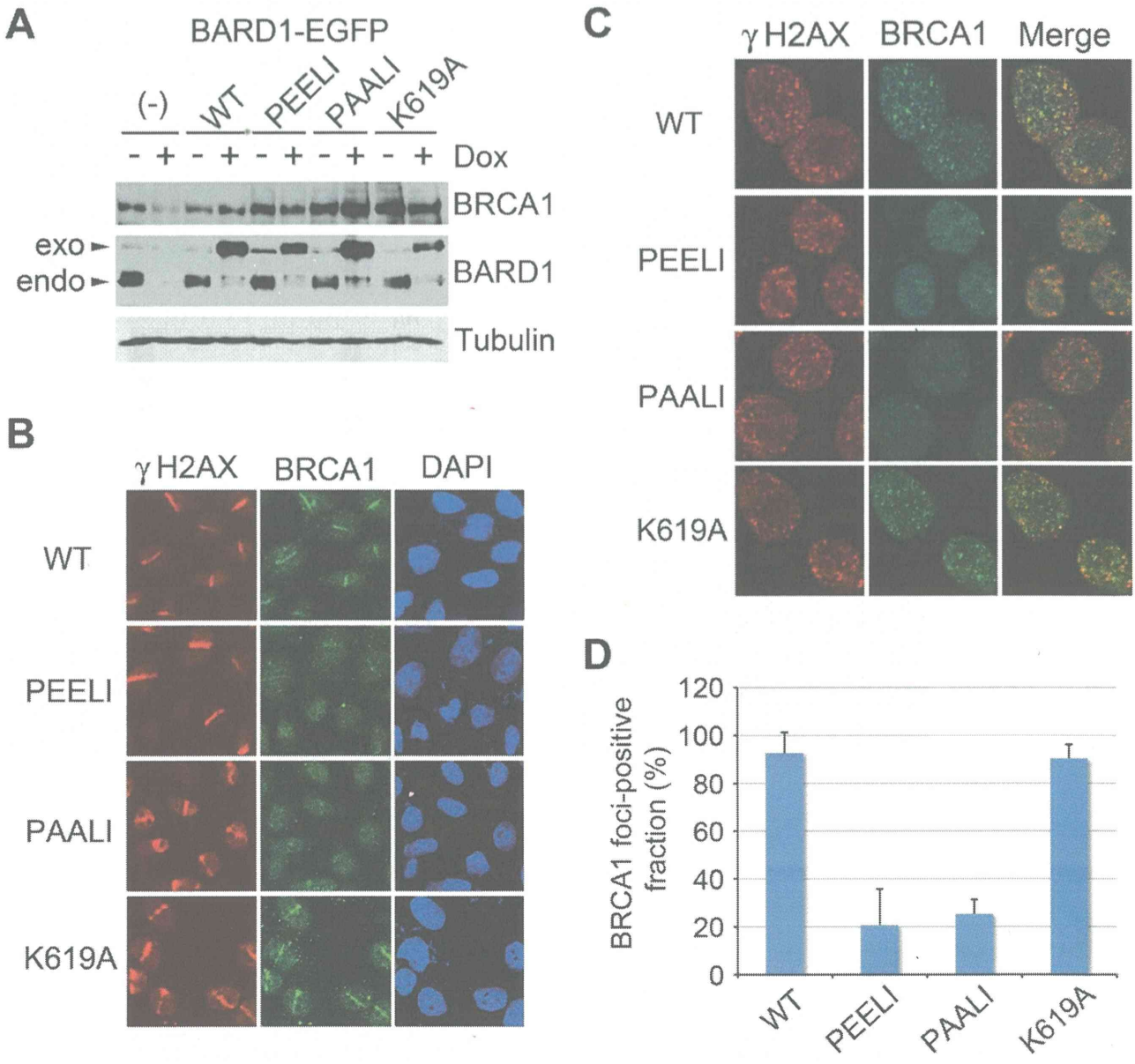
Wu_Fig. 4



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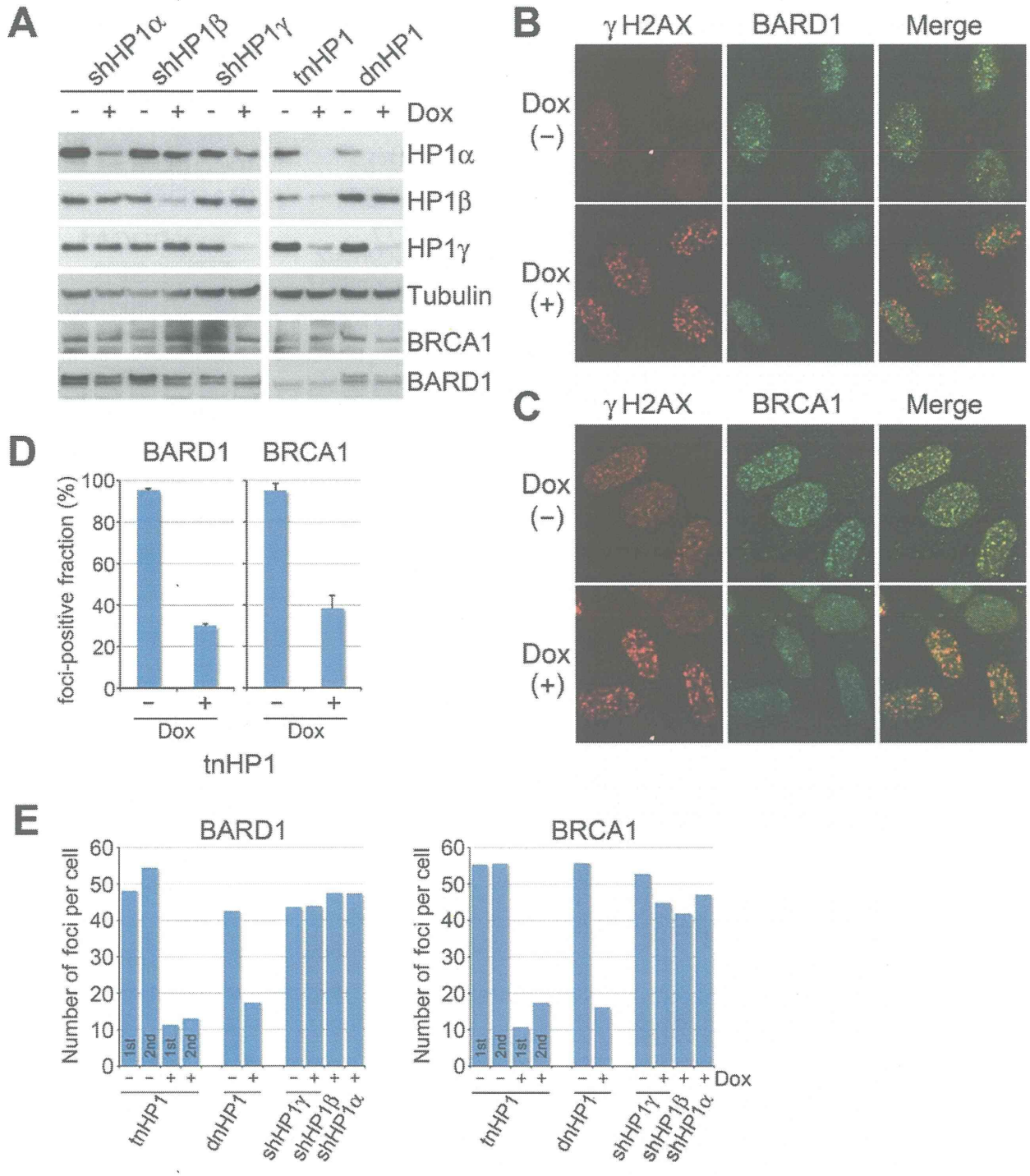
Wu_Fig. 5



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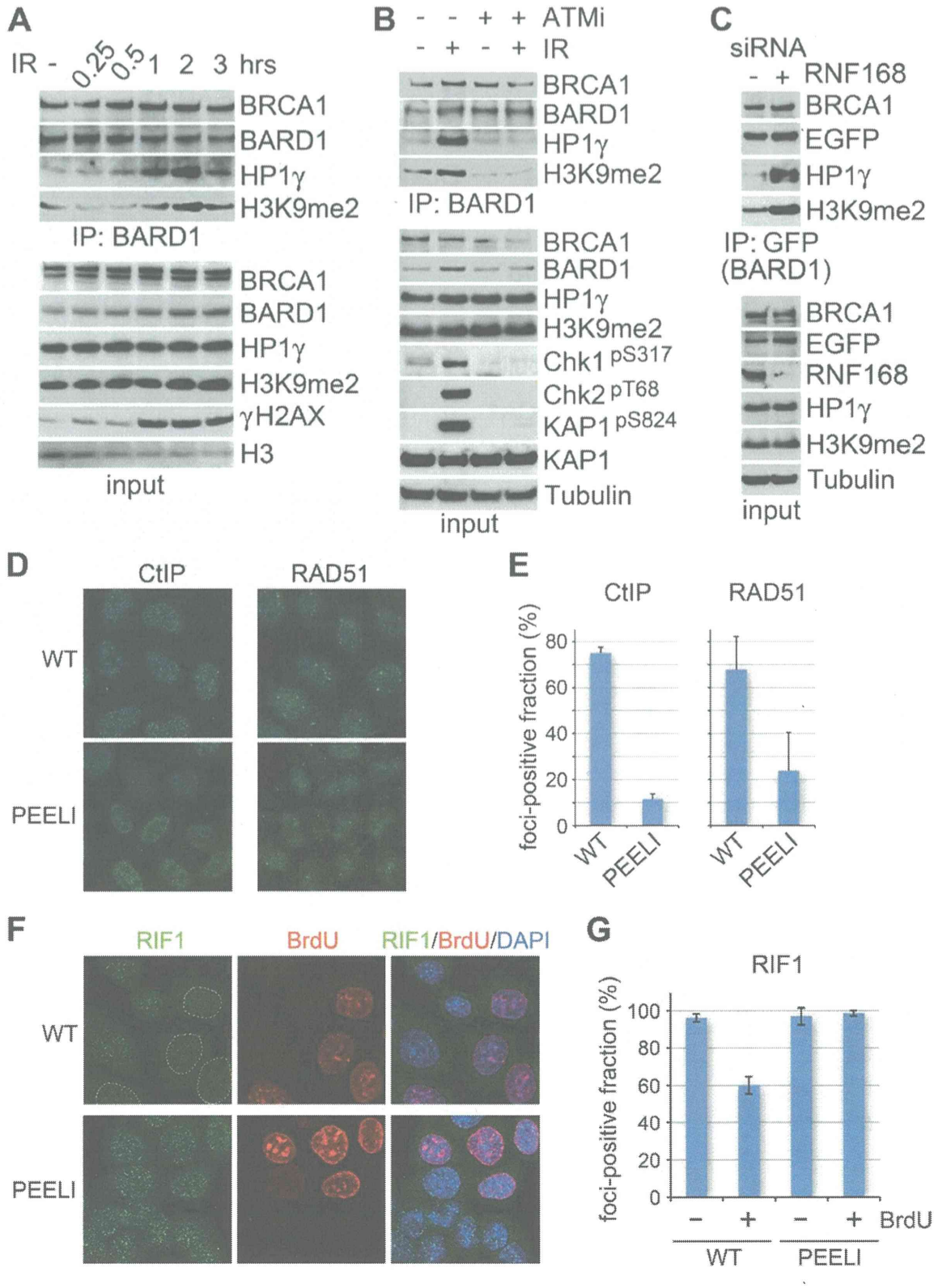
Wu_Fig. 6



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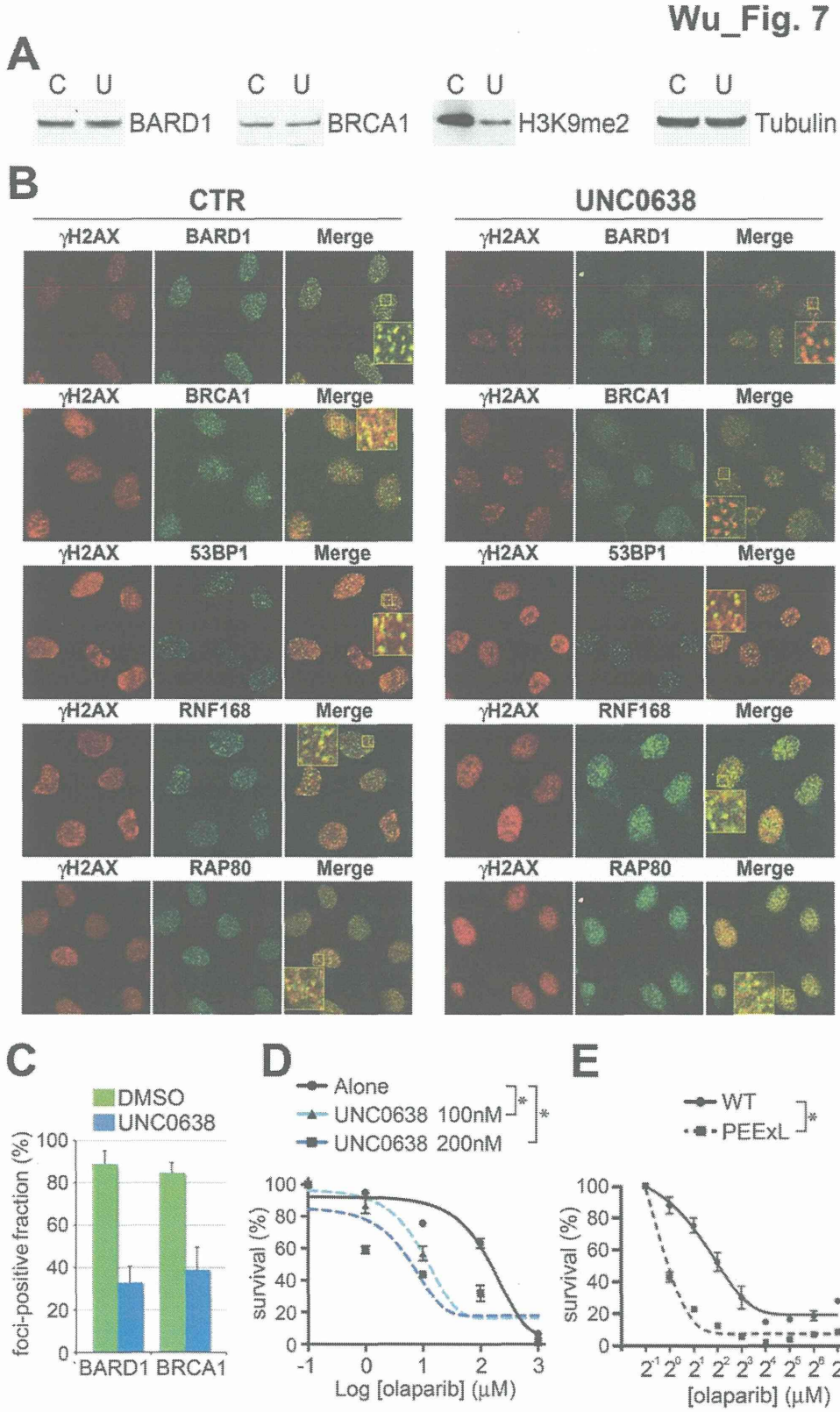
Wu_Fig. 7



AACR American Association for Cancer Research

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Interaction of BARD1 and HP1 is required for BRCA1 retention at sites of DNA damage

Wenwen Wu, Hiroyuki Nishikawa, Takayo Fukuda, et al.

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PLOS | ONE

RESEARCH ARTICLE

LSD1 Overexpression Is Associated with Poor Prognosis in Basal-Like Breast Cancer, and Sensitivity to PARP Inhibition

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Abstract

LSD1, a lysine-specific histone demethylase, is overexpressed in several types of cancers and linked to poor outcomes. In breast cancer, the significance of LSD1 overexpression is not clear. We have performed an *in silico* analysis to assess the relationship of LSD1 expression to clinical outcome. We demonstrate that LSD1 overexpression is a poor prognostic factor in breast cancer, especially in basal-like breast cancer, a subtype of breast cancer with aggressive clinical features. This link is also observed in samples of triple negative breast cancer. Interestingly, we note that overexpression of LSD1 correlates with down-regulation of BRCA1 in triple negative breast cancer. This phenomenon is also observed in *in vitro* models of basal-like breast cancer, and is associated with an increased sensitivity to PARP inhibitors. We propose therefore that high expression levels of the demethylase LSD1 is a potential prognostic factor of poor outcome in basal-like breast cancer, and that PARP inhibition may be a therapeutic strategy of interest in this poor prognostic subtype with overexpression of LSD1.

Introduction

LSD1, lysine specific demethylase 1, removes methyl groups from lysine residues of histone H3, thereby regulating gene expression [1]. For gene repression, LSD1 removes mono- and dimethyl groups from lysine 4 of histone H3 (H3K4) [1]. For gene activation, LSD1 works with androgen/estrogen receptor to remove mono- and dimethyl groups from lysine 9 of histone H3 (H3K9) [2,3]. The control of gene expression by LSD1 has been shown to be vital to multiple processes including organogenesis and stem cell differentiation [4],[5]. In intracellular

processes, it has been suggested that LSD1 promotes cell proliferation, survival and epithelial-mesenchymal transition (EMT) [6]. LSD1 is often overexpressed in malignancies and it is linked to poor clinical outcome in cancers of the lung, liver, colon and esophagus [7–10]. Overexpression of LSD1 has been reported in estrogen receptor negative breast cancer, however it is not known whether LSD1 is a prognostic factor of poor outcome in breast cancer [11].

Breast cancer has been classified into four subtypes based on gene expression profile [12]. Basal-like breast cancer, one of the subtypes, does not display hormonal receptors and human epidermal growth factor receptor 2, HER2, suggesting resistance to hormonal therapy and HER2 antagonism [13]. These tumors display an aggressive clinical course, with high relapse rates [13], and are an important area for the development of new therapeutic strategies. Loss of BRCA1, a familial breast cancer susceptible gene, through mutation or epigenetic dysregulation often leads to tumors with a basal-like phenotype [14]. Recent work has implicated LSD1 in this dysregulation of BRCA1 [6]. Wnt signaling is upregulated in basal-like breast cancer, leading to accumulation of the transcriptional repressor Slug (Snail2) [6]. The accumulated transcription repressor targets LSD1 to promoter region of BRCA1 leading to its downregulation [6]. Thus LSD1 may play a critical role in acquiring poor prognostic phenotype in breast, but the relationship between expression of *LSD1* and the clinical outcome has not been demonstrated to date.

Using bioinformatics tools, we predict that LSD1 expression is linked to poor recurrence free survival of patients with breast cancer, especially in the basal-like breast cancer. We have also investigated the relationship between *LSD1* expression and recurrence free survival in 32 samples of triple negative breast cancer and found that *LSD1* is a prognostic factor of poor clinical outcome. Furthermore, we have shown that *LSD1* overexpression is linked to BRCA1 suppression. Therefore, we propose that PARP inhibitors, a novel class of targeted agents with promising activity in *BRCA* mutant tumors, may be effective therapy for basal-like breast cancers with amplified *LSD1* [15].

Materials and Methods

1.1. Bioinformatic analysis

Gene count data from Breast invasive carcinoma TCGA samples (RNA sequencing) were downloaded from TCGA data portal. PAM50 definitions of intrinsic subtype were used to classify breast cancer into subtypes including basal-like, luminal A, luminal B and HER2 enriched cohorts. The quantitation of mRNA expression was performed using All Complete Tumors of Breast invasive carcinoma (TCGA, Nature 2012) dataset [16]. For the analysis of gene expression, raw counts were normalized by Trimmed Mean of M-values (TMM) using the R package "edgeR" and "calcNormFactors" command. For survival analysis, a set of CEL files (GSE1456) were downloaded from GEO database and normalized by MAS5.0 global mean method. Probe set-based signal intensities were natural log transformed and scaled by adjusting the mean intensity to a target signal value of log500. For survival analysis using KMplot, the data was obtained from kmplot (www.kmplot.com) [17].

1.2. Statistical Analysis

Differential mRNA expression between two or more groups was analyzed by edgeR. For survival analysis, Gehan-Breslow-Wilcoxon tests were performed, as well as cox proportional hazard models. To compare gene expression of BRCA1 in cancer with high or low LSD1, the Mann Whitney U test was performed. Difference were considered to be significant when the two-tailed p-value was < 0.05. Gehan-Breslow-Wilcoxon tests and Mann-Whiney U test were performed using Graphpad prism. Cox proportional hazard models were fitted using the coxph function (located in the survival library in R).