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# TRIM24 links a noncanonical histone signature to breast cancer

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W-W.T. identified ER-interactions, performed molecular biology and IHC studies; Z.W. solved the molecular structures of TRIM24 PHD-Bromo in the free and bound states, and performed ITC binding affinity studies; T.T.Y. performed mutagenesis, ChIP and clonogenic analyses, C-Y.T. performed clonogenic assays; K.C.A. performed bioinformatic analyses; W.X. performed analyzed patient samples; X.S. performed peptide array analyses; S.W., D.S. and W.F. performed and analyzed FP experiments; O.G., B.A., W.P., W.F., M.-C. H., D.J.P. and M.C.B. discussed studies; D.J.P. and M.C.B. designed structural and functional studies, analyzed data and wrote the paper. W-W.T. and Z.W. contributed equally to this work. All authors discussed and commented on the manuscript.

**AUTHOR INFORMATION**

**Coordinates and Sequencing Data Deposition**

The X-ray coordinates of TRIM24 PHD-Bromo in the free state and when bound to H3(1–10)K4, H3(13–32)K23ac, H3(23–31)K27ac and H4(14–19)K16ac peptides have been deposited in the Protein Data Bank (PDB) under accession numbers of 3O33, 3O37, 3O34, 3O35 and 3O36 respectively. ChIP-sequencing files and data are deposited at the NCBI Gene Expression Omnibus (GEO) site as accession number GSE24166.

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## Abstract

Recognition of modified histone species by distinct structural domains within "reader" proteins plays a critical role in the regulation of gene expression. Readers that simultaneously recognize histones with multiple marks allow transduction of complex chromatin modification patterns into specific biological outcomes. Here, we report that chromatin regulator TRIM24 functions as a reader of dual histone marks via tandem Plant Homeodomain (PHD) and Bromodomain (Bromo). The three-dimensional structure of TRIM24 PHD-Bromo revealed a single functional unit for combinatorial recognition of unmodified H3K4 (H3K4me0) and acetylated H3K23 (H3K23ac) within the same histone tail. TRIM24 binds chromatin and estrogen receptor to activate estrogen-dependent genes associated with cellular proliferation and tumor development. Aberrant expression of TRIM24 negatively correlates with survival of breast cancer patients. The PHD-Bromo of TRIM24 provides a structural rationale for chromatin activation via a noncanonical histone signature, establishing a new paradigm by which chromatin readers may influence cancer pathogenesis.

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Post-translational modifications of histones occur in combinations that must be faithfully translated by effector proteins, or histone readers[1–4]. The lexicon of histone modifications may be highly context-dependent, influenced by inductive signaling, cellular milieu and target gene status[4]. Misinterpretation or imbalance in this hierarchal arrangement has dire consequences for cellular homeostasis, leading to developmental problems, hereditary disease or tumor development[5]. Linked histone reader modules, such as tandem PHD finger and Bromodomain, occur frequently in histone interacting proteins but little is known about their mechanisms of action. Combinatorial readout of histone post-translational modifications (PTM's) may enhance binding between spatially separated histone marks, or even create communication links between domains or members of the complex[3]. Individually, proteins with Bromodomains, e.g. TAF1 and BDF1, associate with acetylated lysines with broad specificity[6,7], while PHD-containing proteins are less predictable in their interactions[1–4]. The PHD fingers of BHC80 and AIRE interact with unmethylated H3K4 (H3K4me0)[8,9], while other previously reported PHD finger domains bind methylated proteins as modifiers of histones or subunits of chromatin remodeling, co-activator or co-repressor complexes[1–4,10–17].

PHD-finger proteins and their dysregulation are linked to a broad spectrum of human diseases, underscoring an essential role in homeostasis[5]. Recently, aberrant localization of a JARID1A PHD finger-fusion protein was shown as directly causal in transformation and development of hematopoietic malignancy, a process requiring fusion protein recognition of H3K4me3 via the JARID1A PHD finger[18]. Here, we present evidence that a multi-functional protein, TRIM24, which is an E3-ubiquitin ligase that targets p53[19] and is

broadly associated with chromatin silencing[20], relies on tandem PHD finger and Bromodomain (designated PHD-Bromo) to recognize specific, combinatorial histone modifications and activate estrogen-dependent genes associated with cellular proliferation and tumor development. Genome-wide analysis of chromatin interactions shows estrogen-dependent binding of TRIM24 and estrogen receptor alpha (ERα) at sites that paradoxically exhibit estrogen-activated loss of H3K4me2 and gain of histone acetylation. Importantly, aberrant over expression of TRIM24 in breast cancer patients is frequent and directly correlated with poor survival.

## TRIM24 PHD-Bromo binds N-terminal H3 tail

TRIM24 belongs to the TRIM/RBCC protein family, characterized by a conserved, amino (N)-terminal tripartite motif: a RING domain, B-box zinc-fingers, a coiled-coil region, as well as variable carboxy (C)-terminal domains[21],[22]. TRIM24 was originally identified as Transcriptional Intermediary Factor (TIF) 1α, a ligand-dependent, co-repressor of retinoic acid receptor that interacts with multiple nuclear receptors *in vitro* via an LXXLL motif[23]. In addition to its LXXLL motif and RING domain, TRIM24 has a C-terminal, PHD-Bromo (Fig. 1a), which likely recognizes histones or non-histone proteins with specific combinations of post-translational modifications.

Protein sequence alignment of the PHD fingers of TRIM24 and BHC80 with ING1, a PHD domain that recognizes H3K4me3[24],[25], showed TRIM24 as highly similar to BHC80 with conservation of residues critical for BHC80-H3K4me0 interactions[8] (Supplementary Fig. 1a). Accordingly, we found that full-length TRIM24 interacts with histone proteins specifically through its PHD-Bromo (Supplementary Fig. 1b). Binding of the TRIM24 PHD-Bromo to histone peptide arrays occurs at unmodified H3 (residues 1–21), methylated H3K9 (H3K9me) and acetylated H3K9/K14 peptides, but not methylated H3K4 residues (Supplementary Fig. 1c). Similarly, TRIM24 PHD finger and PHD-Bromo bind unmodified histone H3 (residues 1–21) but not methylated H3K4, similar to BHC80 but unlike ING1, which preferentially binds to H3K4me peptides (Fig. 1b and Supplementary Fig. 1d). GST-pulldown assays with native histones confirmed that TRIM24 PHD finger, Bromodomain, PHD-Bromo and the BHC80 PHD fail to bind to native histone H3 with K4 trimethylation (H3K4me3) but tolerate H3K9me2 modification (Fig. 1c and Supplementary Fig. 1e). Isothermal titration calorimetry (ITC) based binding assays established that the PHD-Bromo binds unmodified H3(1–15)K4 with a $K_D$ of 8.6 μM, while methylation of H3K4 greatly decreases binding affinity of TRIM24 and H3 peptides (Fig. 1d and Supplementary Table 2). These results suggest that TRIM24 PHD-Bromo interacts with the N-terminal tail of histone H3 but specific PTM's, *e.g.* methylation of H3K4, interfere with this interaction.

## Structural basis of H3 readout by TRIM24

We have determined the three-dimensional crystal structure of the PHD-linker-Bromo segment (residues 824–1006) of human TRIM24 in free and histone peptide bound states. The overall structure of TRIM24 PHD-Bromo in the free state demonstrates that PHD and Bromodomain interact extensively and form an integrated structural unit (747 $Å^2$ of contact surface), connected by a long linker and stabilized by a network of hydrogen bonding and

hydrophobic interactions (Fig. 2a, Supplementary Fig. 2 and Supplementary Table 1). The TRIM24 PHD finger residues 824–871 adopt the typical PHD finger 'cross-braced' topology stabilized by a pair of coordinated zinc ions, which together with residues 872–884 from the linker region form an extended TRIM24 PHD domain. The TRIM24 Bromodomain adopts the typical left-handed four-helical bundle characteristic of other members of this family.

The 2.0 Å co-crystal structure of TRIM24 PHD-Bromo and unmodified H3(1–10)K4 peptide (Supplementary Table 1 and Supplementary Fig. 3a) showed that the first 9 residues of bound H3 peptide are positioned within a surface groove of the PHD finger (Fig. 2b and Supplementary Fig. 3b). The R2 to Q5 segment of bound H3 peptide forms an anti-parallel β-sheet with the E837 to C840 segment of the PHD finger, while the T6 to K9 segment of bound H3 peptide contacts the N834 to G836 segment of the PHD finger. The side chain of R2 is hydrogen-bonded with the backbone carbonyl of C841. The side chain of C840 is positioned in-between the side chains of R2 and K4, with the C840W mutation losing its ability to bind unmodified H3K4 peptide ($K_D$ > 400 μM, Supplementary Table 2 and Supplementary Fig. 4).

The unmodified lysine ammonium group of H3K4 forms two direct hydrogen bonds with backbone carbonyl oxygens of N825 and E826 (Fig. 2b). In addition, the proximally positioned D827 forms a stabilizing salt bridge with the unmodified lysine, consistent with the observation of impaired binding between D827A mutant and unmodified H3K4 peptide ($K_D$ = 133 μM, Supplementary Table 2). Methylation of H3K4 would create steric clashes with residues lining the binding pocket, disrupt the salt bridge interaction with D827, and impair hydrogen bonding with N825 and E826, thereby providing a structural explanation for the unmodified H3K4 preference of TRIM24 PHD-Bromo.

## TRIM24 Bromodomain is H3K23ac-specific

Both sequence and structure-based alignments indicate that TRIM24 Bromodomain is an acetyllysine reader. Peptide pulldown assays and NMR titration measurements suggest that TRIM24 Bromodomain interacts with H3 peptides with K23 or K27 acetylation and several acetylated H4 peptides (Supplementary Fig. 5a and 5b). ITC studies establish that TRIM24 PHD-Bromo specifically binds to the H3(13–32)K23ac peptide with a dissociation constant ($K_D$ = 8.8 μM; Supplementary Table 2), comparable to tetra-acetylated H4 peptide and double Bromodomain modules of TAF1 or BDF1.

We solved the 1.9 Å crystal structure of the complex of TRIM24 PHD-Bromo and H3(13–32)K23ac peptide (Supplementary Table 1 and Supplementary Fig. 6a). Residues 23–27 of the bound H3(13–32)K23ac peptide exhibit sequence-specific interactions with TRIM24 Bromodomain (Fig. 2c and Supplementary Fig. 6b). The acetyllysine side chain forms a direct hydrogen bond with the side chain of conserved N980. Acetyllysine recognition constitutes the binding determinant, as double mutant F979A/N980A loses most of the binding affinity for the H3(13–32)K23ac peptide (Supplementary Table 2).

ITC studies establish that H3(1–20)K9ac, H3(1–19)K14ac and H3(13–32)K27ac bind non-specifically to the TRIM24 Bromodomain ($K_D$ ~ 200 μM Supplementary Table 2). The

crystal structure of the complex of TRIM24 PHD-Bromo with H3(23–31)K27ac peptide (Supplementary Table 1) revealed a single intermolecular hydrogen bond between the side chains of K27ac and N980, while other histone residues did not show any direct intermolecular contacts with the Bromodomain (Supplementary Fig. 7), consistent with weak binding affinity of H3(13–32)K27ac peptide. The structure of TRIM24 PHD-Bromo bound to H4(14–19)K16ac peptide containing the conserved interaction between K16ac and N980 side chains is shown in Supplementary Fig. 8.

The structures of TRIM24 PHD-Bromo complexes with acetyllysine-containing histone peptides show that acetyllysine invariantly inserts into a pre-formed acetyllysine-binding pocket of the bromodomain. With the acetyllysine as an anchor, flanking residues determine sequence specificity of acetyllysine peptides for the TRIM24 bromodomain. The H3(13–32)K23ac peptide both fits better within the cleft between ZA and BC loops, and shows sequence-specific interactions with TRIM24 Bromodomain spanning K23ac to K27, creating much higher affinity for the TRIM24 Bromodomain, versus other acetyllysine-containing peptides.

## Combinatorial readout by TRIM24 PHD-Bromo

Superimposition of the above structures of complexes revealed that H3K4 and H3K23ac peptides are aligned in the same direction on the surface of the TRIM24 PHD-Bromo (Fig. 2d). The distance between the Cα of H3K9 and the Cα of H3K23ac is 25.5 Å, which allows one H3 peptide containing both unmodified H3K4 and H3K23ac to simultaneously target the PHD and Bromodomain binding sites on TRIM24 PHD-Bromo.

By contrast, H3K4 and H3K27ac (or H4K16ac) peptides are aligned in opposite directions on the surface of TRIM24 PHD-Bromo (Supplementary Fig. 9), which indicates that the TRIM24 PHD-Bromo requires two histone tails, either within a single nucleosome or from an adjacent pair of nucleosomes, to simultaneously bind H3K4 and H3K27ac (or H4K16ac).

To test the effect of combinatorial readout of TRIM24 PHD-Bromo for histone H3 bearing unmodified K4 and acetylated K23 dual marks, we synthesized longer H3(1–33) peptides bearing both unmodified K4 and acetylated K23 marks. For controls, we used H3(1–33)K4me3K23ac, as well as H3(1–33)K4 peptides that have only one effective histone mark for specific TRIM24 PHD-Bromo recognition. Based on ITC binding assays, TRIM24 PHD-Bromo showed an approximately 90-fold higher binding affinity for H3(1–33)K4K23ac peptide (Fig. 2e, $K_D$ = 0.096 μM) compared to the shorter H3(1–15)K4 peptide bearing only unmodified K4 ($K_D$ = 8.6 μM) or for the H3(13–32)K23ac peptide bearing only acetylated K23 marks ($K_D$ = 8.8 μM). Without acetylation on K23, the binding for H3(1–33)K4 is 24-fold weaker (Fig. 2e; $K_D$ = 2.3 μM); when K4 is tri-methylated, the binding for H3(1–33)K4me3K23ac is 6-fold weaker (Fig. 2e; $K_D$ = 0.56 μM). Similarly, mutants that disrupt either the PHD finger binding pocket (C840W) or bromodomain binding pocket (F979A/N980A) also decreased binding for H3(1–33)K4K23ac peptide by 6–7 fold (Fig. 2e and Supplementary Table 2).

By fluorescence polarization (FP) based measurement, wild-type TRIM24 PHD-Bromo also showed strong binding affinity for H3(1–33)K4K23ac peptide ($K_D$ = 0.185 μM); peptides

trimethylated at K4 or without acetylation at K23 displayed 13–23 fold weaker interaction (Fig. 2f and Supplementary Table 3). Mutation on the PHD finger binding pocket (C840W) or the Bromodomain binding pocket (F979A/N980A) showed similar decrease in binding affinities (Fig. 2f and Supplementary Table 3). These binding data strongly support our structural results, which indicate that unmodified H3K4 and acetylated H3K23 are a pair of natural histone marks targeted by TRIM24 PHD-Bromo that can be read in a combinatorial manner on a single histone peptide. This combinatorial readout can greatly increase the recruitment of TRIM24 to nucleosomes bearing these two marks.

## TRIM24 and ERα recruitment to chromatin

Combinatorial histone modifications of unmethylated H3K4 alongside acetylated lysines have no straightforward interpretation by the paradigms of chromatin modification and regulated activation or repression of transcription. We considered a model where TRIM24 regulates gene expression by specific binding to chromatin with non-canonical combinations of PTM's, and focused on co-regulation of ERα, as in vitro interactions between TRIM24 and nuclear receptors, including ERα, are ligand-dependent (Supplementary Fig. 10 and [26]), and ligand-activated, ER-response elements (ERE's) are notably independent of H3K4me2 and H3K4me3 modifications[27,28]. We used ChIP and sequential ChIP analyses of ERα-positive, MCF7 breast cancer cells to assess whether TRIM24 is recruited with ERα to specific ERE's of the *GREB1*, *PR* and *pS2/TFF1* genes (Fig 3a, 3b and Supplementary Fig. 11). Estrogen-activated recruitment occurs robustly within 15 minutes, and by six hours yields a 7-fold increase of ERα and 6-fold increase of TRIM24 binding at the *GREB1* distal ERE, ~40 Kb upstream of the transcription start site (Fig. 3a). ChIP analysis of H3K4me2/3 after estrogen treatment indicates that quantified H3K4me2 and H3K4me3 levels decreased at distal ERE sites (Supplementary Fig. 12 and [27]) and, when normalized for nucleosomal occupancy, decreased or are unchanged at distal ERE's (Fig. 3c and Supplementary Fig. 13). Importantly, TRIM24 is recruited in the absence of changes in H3K4 methylation. In contrast, H3K23ac, H3K27ac and H4ac, which are targeted by the TRIM24 Bromodomain, are enriched at both distal and proximal ERE's after $E_2$-addition (Fig. 3d). These findings suggest that TRIM24 interacts with ERα and chromatin lacking H3K4 methylation but enriched in lysine acetylation, as suggested by our structural analyses, in response to estrogen.

These findings stand in contrast to a model of chromatin accessibility at ER binding sites, facilitated by FOXA1 and H3K4me2 enrichment in response to estrogen treatment[29], but are in agreement with findings that H3K4me3 is not present at a majority of distal ERE regions[28]. We evaluated global chromatin- association of TRIM24, ERα and H3K4me2, by ChIP and deep sequencing of antibody-enriched DNA fragments (ChIP-seq). These analyses revealed binding of TRIM24 and ER at more than 10,000 sites genome-wide; half of which, in each case, are estrogen-dependent (Fig. 3e and Supplementary Fig. 14a). Shared target sites of ERα and co-regulator TRIM24 increase dramatically (eight- fold) in response to estrogen (Supplementary Fig. 14b), are highly enriched (p- value<0.001) at genes regulated by estrogen[30] (Supplementary Fig. 14c), and function in cell cycle, kinase activity and signal transduction (DAVID analyses[31], Supplementary Table 4). Biological pathway analysis (IngenuityR Systems, www.ingenuity.com) revealed that multiple gene targets of TRIM24

are associated with breast cancer (Supplementary Tables 5 and 6). The number of target sites shared by TRIM24 and ERα (1677 sites) is similar to ERα and FOXA1[29], with little overlap among all three (263 sites) (Supplementary Fig. 14b). Consistent with our structural analyses, TRIM24 binding occurs globally at sites depleted of H3K4me2 (Fig. 3f and Supplementary Figs. 14d and 15). Thus, ERα-regulated genes may be divided into multiple classes, defined by specific co-regulators and their dependence on H3K4 methylation.

## TRIM24 is over expressed in breast cancer

Depletion of TRIM24 caused a significant decrease in ERα-mediated activation of *GREB1*, *PR* and *pS2* gene expression (Fig. 4a and Supplementary Fig. 16a). Importantly, re-introduction of wild type (WT), but not PHD finger mutant (C840W), TRIM24 fully restored ERα-mediated transcription activation (Fig. 4b), and enabled ERα-response at lower levels of hormone (Fig. 4c). Decreased ERα-mediated activation is due to loss of TRIM24-dependent ERα-interactions with chromatin (Fig. 4d and Supplementary Fig. 17), without alternation of ERα expression (Supplementary Fig. 16b). H3K4me2/3 levels at the distal ERE of *GREB1* lack hormone responsiveness and are TRIM24-independent (Fig. 4d and Supplementary Fig. 16c). In contrast, nucleosomal occupancy at ERE's is increased alongside decreased acetylation of H4, H3K23 and H3K27, reflecting loss of ERα-activated chromatin structure (Fig. 4d and Supplementary Fig. 16c).

Strikingly, depletion of TRIM24 led to reduced survival and proliferation of tumor-derived breast cancer cells, and is highly additive with 4-OH-tamoxifen, an inhibitor of ERα[32] (Fig. 5a). We immunostained tissue samples from a breast cancer patient cohort to assess the impact of TRIM24 expression in breast cancer survival (Fig. 5b). In 128 cases of nonmetastatic breast cancer, expression of TRIM24 fell into four classes: N- and N+, undetectable to low level in few foci (29%); N++, abundant foci with expression in nuclear and cytoplasmic compartments (20%); and, N+++, abundant foci with high expression in nuclei (51%). Over expression of TRIM24 (+++, ++) is clearly correlated with poor patient survival, independent of ER-status (Fig. 5c and Supplementary Table 7).

## Discussion

Our identification of the PHD-Bromo as a reader of H3K4me0 and H3K23ac within a single histone tail or H3K4me0 and noncontiguous acetylated lysines suggests that TRIM24 may have multiple roles in chromatin regulation[20]. TRIM24 is a co-activator of ERα at distal ERE's, a platform well suited for stable interactions with TRIM24 PHD-Bromo. ERα recruits histone acetyltransferases, *e.g.* CBP/p300, GCN5 and P/CAF[33], to acetylate histones. LSD1 (KDM1), a biochemically and structurally characterized demethylase for H3K4me2/1[34],[35] and androgen-regulated demethylase of H3K9me[36], is resident[37] or rapidly recruited[27] to ERE's where H3K4 remains depleted of methylation even with estrogen activation (Fig 3c, Supplementary Fig. 18 and [28]). These parallel processes establish a combinatorial histone signature with high affinity for TRIM24 binding to chromatin.

Aberrant expression of TRIM24 may promote tumor development and progression by multiple mechanisms of dysfunction. TRIM24 is a potent co- activator of ERα, which is

associated with cellular proliferation and neoplasia in breast cells[38],[39], and a negative regulator of p53 stability[19]. TRIM24 is a target of chromosomal translocations to form oncogenic fusion proteins in acute promyelocytic leukemia[40], papillary thyroid carcinoma[41] and myeloprolferative syndrome[42]. Here, we show that TRIM24 expression is directly correlated with poor patient survival in both ER-positive and ER-negative breast cancer. These results suggest that TRIM24 is a dual domain, histone reader with considerable potential as a therapeutic target in multiple cancers.

## METHODS SUMMARY

Wild-type and mutant forms of TRIM24 PHD-Bromo were expressed in *E.coli* and purified to homogeneity. Histone biotinylated peptides or purified histone proteins were incubated with GST-proteins, and bound proteins detected by immunoblotting. All crystals were obtained by hanging-drop method at 20°C, structures were solved by molecular replacement method and refined with cycled model building and refinement procedures. Histone peptides with or without biotin labeling were used for ITC binding. Fluorescein-labeled peptides were used for fluorescence polarization analysis. Stable shControl and shTRIM24 MCF7 cells were maintained with 2.5 μg/mL puromycin and, for hormone treatment, were grown in hormone-free media for 96 h prior to addition of ethanol or 10 nM estradiol (Sigma) for indicated times. Global expression analyses and calculation of enrichment of shared TRIM24 and ERα binding at estrogen- regulated genes[30] were determined, and validated by real-time RT-PCR. Surgical specimens of breast cancer from 128 nonmetastatic patients were immunostained for TRIM 24 (TRIM24 antibody, Proteintech Group, Inc., Chicago, IL), and scored by subcellular localization (nuclear, N), staining intensity, and fraction of positive staining. The overall survival after surgery was plotted by the Kaplan-Meier method[43].

Full methods are available online.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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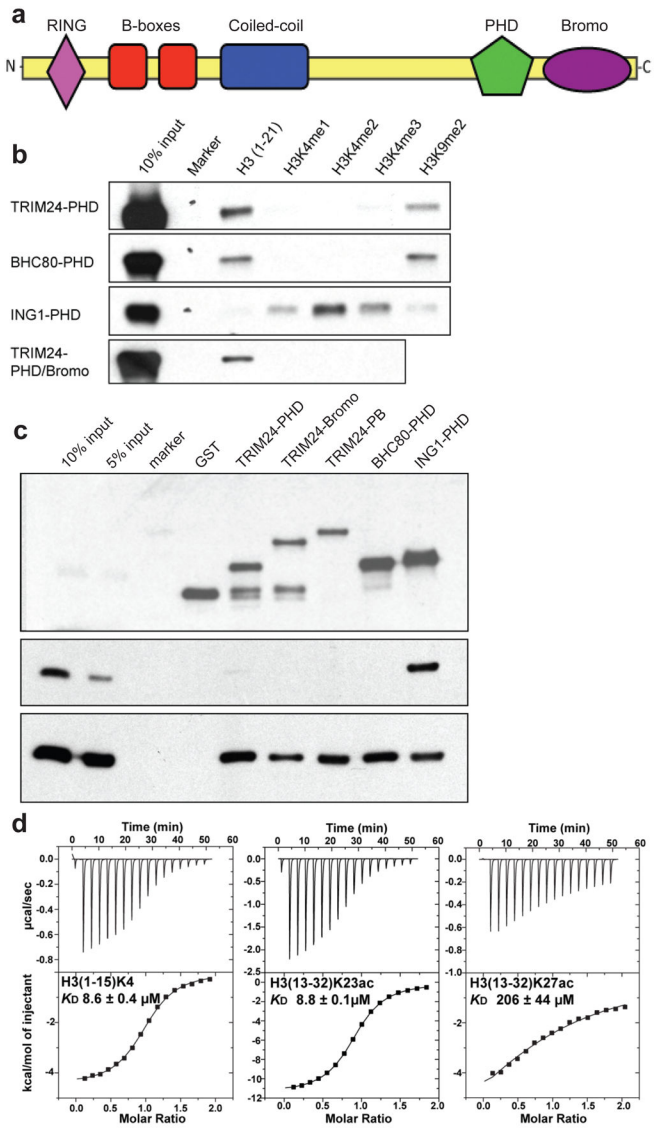


**Figure 1. TRIM24-PHD finger interacts with unmethylated H3K4**

**a,** Diagram of TRIM24 protein domains. **b,** Biotinylated peptide pulldowns: recombinant PHD fingers and histone peptides. **c,** GST-pulldowns: recombinant proteins and native histone proteins. **d,** ITC titration: binding of TRIM24 PHD-Bromo with histone peptides.

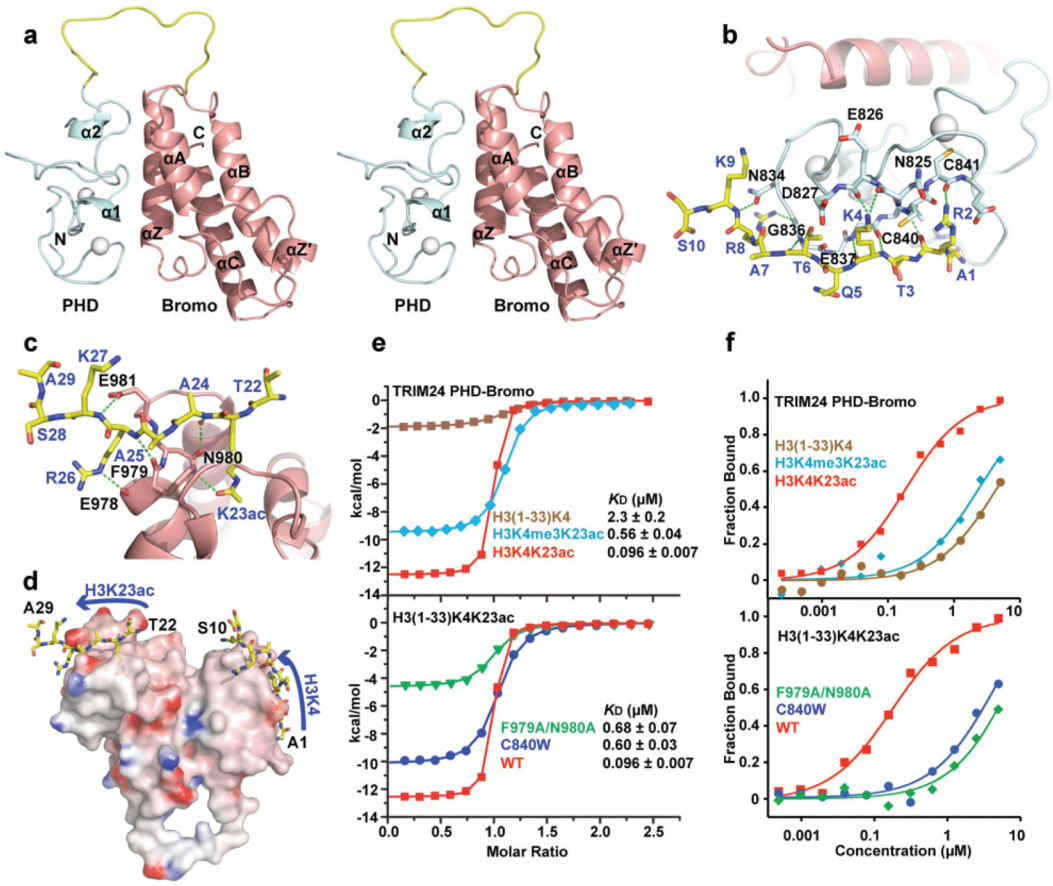


**Figure 2. TRIM24 PHD-Bromo simultaneously binds H3K4me0 and acetylated histone lysines**

**a,** Stereo view of the crystal structure of TRIM24 PHD-Bromo in the free state. **b,** Detailed interactions between PHD of TRIM24 PHD-Bromo and H3(1–10)K4 peptide. **c,** Detailed interactions between Bromodomain of TRIM24 PHD-Bromo and H3(22–29)K23ac peptide. **d,** Positioning of H3(1–10)K4 and H3(13–32)K23ac peptides on the surface of TRIM24 PHD-Bromo based on structural information **e** and **f,** ITC (as in **e**) or fluorescence polarization (FP) (as in **f**) based binding curves of wild-type (WT) or mutant forms of TRIM24 PHD-Bromo with H3(1–33) peptides bearing different combination of modifications. Dissociation constants ($K_D$) derived from ITC experiments are given as inserts.

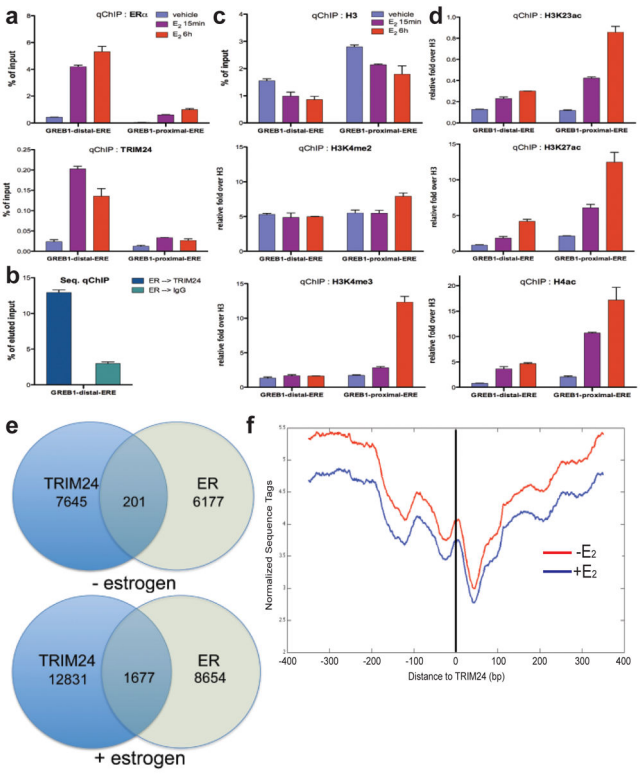


**Figure 3. TRIM24 is recruited with ERα to ERE sites depleted of H3K4me2**

**a,** ChIP of ERα and TRIM24 at ERE's of *GREB1*, 15 min and 6 h estradiol ($E_2$). Vehicle: EtOH. **b,** Sequential-ChIP: ERα and TRIM24, 6 h $E_2$. **c, d,** ChIP for H3 and histone modifications, 15 min and 6 h $E_2$, normalized for H3. Each bar represents averaged results, n=3 biological replicates, assayed 3 times each; error bars show standard deviations. **e,** Genome wide TRIM24 and ERα binding sites in MCF7 cells, $-E_2$ or $+E_2$. Two independent experiments analyzed. **f,** Normalized genome wide H3K4me2 within a window of 800 bp, centered at TRIM24 binding sites (designated as 0), $+E_2$ (blue line) or $-E_2$ or (red line).

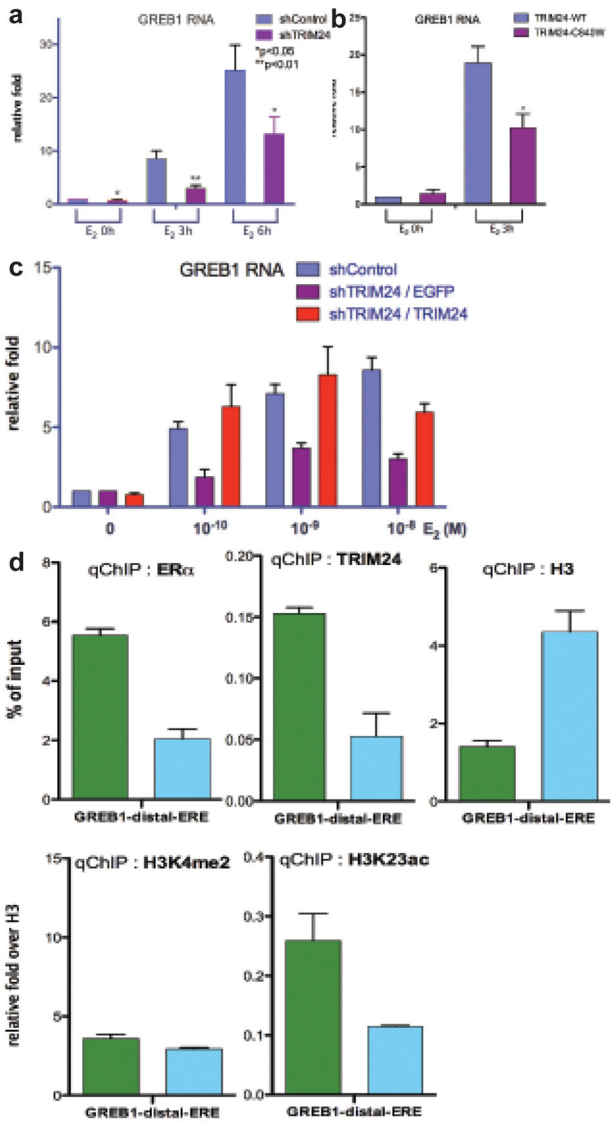


**Figure 4. TRIM24 functions as a co-activator and stabilizes ERα-chromatin interactions**

**a,** Stable shControl and shTRIM24 MCF7 cells +/− $E_2$. **b,** TRIM24-WT and TRIM24-C840W expressed in stable shTRIM24 MCF7 cells +/− $E_2$. **c,** shControl and shTRIM24 MCF7 cells, $E_2$ range. TRIM24-WT or EGFP control expressed in shTRIM24 MCF7 cells. (in **a, b** and **c**) *GREB1* RNA levels normalized to *GAPDH;* untreated shControl MCF7 set as one. Each bar is an average of 3 biological replicates, 3 independent RT-PCR assays of each; error bars show standard deviation. **d,** ChIP of ERα and TRIM24, histone H3 and histone modifications, 6 h $E_2$, shControl and shTRIM24 MCF7 cells. Histone modifications normalized for H3 recovery. Each bar represents averaged results, n=3 and 3 assays of each; error bars show standard deviation.

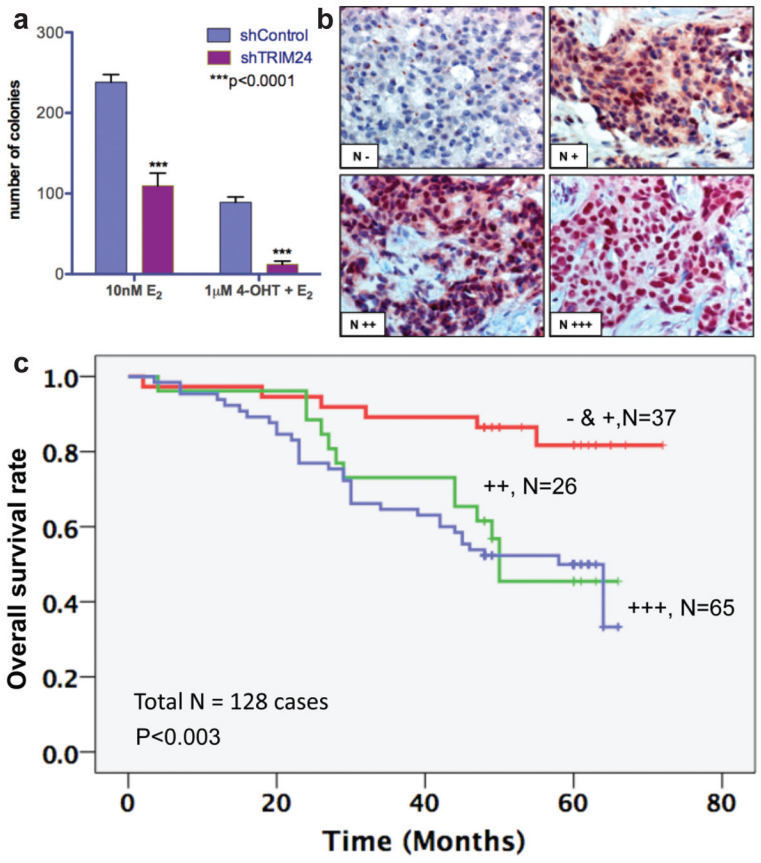


**Figure 5. Aberrant expression of TRIM24 correlates with poor survival of breast cancer patients**

**a,** shControl and shTRIM24 MCF7 cells plus $E_2$ or $E_2$ plus 4-hydroxytamoxifen, as indicated. Each bar represents the averaged results for three independent colony formation assays in triplicate plates; error bars show standard deviation. **b,** Immunohistochemistry: 128 surgical specimens of breast cancer immunostained for TRIM24: subcellular localization (N) and staining intensity (strong, +++; moderate, ++; weak or slightly above background, +; none, −). **c,** The overall survival rate of 128 patients with nonmetastatic disease, classified by TRIM24 expression (as in b), plotted by the Kaplan-Meier method.