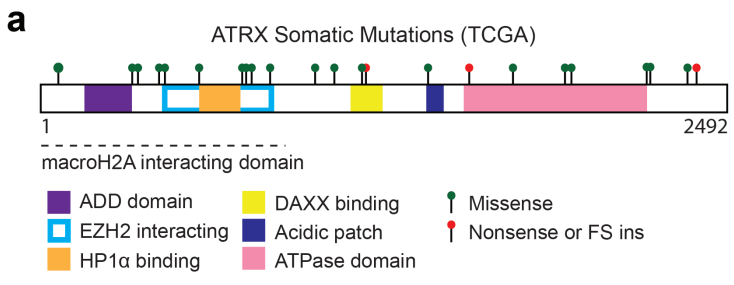
Supplemental Figures



Case ID	AA Change	Type	Chr	Start Pos	End Pos	Ref	Var
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TCGA-D3-A3C8	*H758Y*	Missense	chrX	76938476	76938476	G	A
TCGA-D3-A3MV	*S334F*	Missense	chrX	76939747	76939747	G	A
TCGA-EE-A2GC	*D1263E*	Missense	chrX	76931741	76931741	G	T
TCGA-EE-A2GE	*A1790fs*	FS ins	chrX	76874353	76874354	-	CACA
TCGA-EE-A2GM	*D1487N*	Missense	chrX	76907702	76907702	C	T
TCGA-EE-A2GO	*E2060K*	Missense	chrX	76845343	76845353	C	T
TCGA-EE-A2MJ	*S342F*	Missense	chrX	76939723	76939723	G	A
TCGA-EE-A3JA	*K1936R*	Missense	chrX	76855029	76855029	T	C
TCGA-EE-A3JI	*S925F*	Missense	chrX	76937974	76937974	G	A
TCGA-ER-A19P	*M1005I*	Missense	chrX	76937733	76937733	C	G
TCGA-ER-A19P	*R2150I*	Missense	chrX	76814195	76814195	C	A
TCGA-ER-A19P	*E2351**	Nonsense	chrX	76776901	76776901	C	A
TCGA-ER-A19S	*L639F*	Missense	chrX	76938831	76938831	C	G
TCGA-ER-A19S	*E641K*	Missense	chrX	76938827	76938827	C	T
TCGA-ER-A2NG	*E2333K*	Missense	chrX	76776955	76776955	C	T
TCGA-ER-A3ET	*E1268**	Nonsense	chrX	76931728	76931728	C	A
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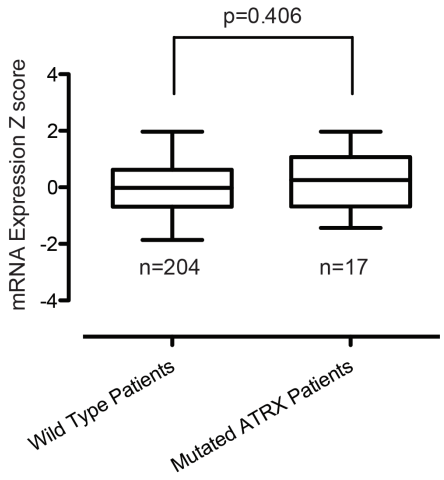

Supplemental Figure S1. ATRX status in cutaneous melanoma as reported by TCGA. (**a**) Table illustrating reported ATRX mutations (17/228 melanoma tumors; mutation rate = 7.5%). Four tumors displayed multiple ATRX mutations (23 mutations identified in 17 tumors). Schematic (top) depicts approximate location of mutations within the ATRX protein product. (**b**) Plot showing ATRX mRNA expression (from RNA-seq) of ATRX wild type vs. mutated patients. The Z score was calculated by cBioPortal by normalizing the mRNA expression values to the average of the reference samples. Statistical significance was derived using unpaired Student's t-test, p-value indicated. All patient samples utilized for this analysis have mRNA expression and sequencing data available at http://www.cbioportal.org.

Supplemental Materials and Methods

Immunohistochemistry and statistical analysis.

Immunohistochemistry was performed as previously described (Kapoor *et al.,* 2010). Briefly, Institutional Review Board (IRB) approved FFPE specimens were obtained from Icahn School of Medicine at Mount Sinai Division of Dermatopathology (project number HSD08-00565) and Mount Sinai Biorepository Cooperative. Tumor sections were incubated with anti-ATRX antibody (Sigma, St. Louis, MO, Cat # HPA001906, 1:650). Slides were scored by two independent dermatopathologists in a blinded fashion and quantified for number of positively stained cells as well as intensity of the stain. The number of cells with positive nuclear staining was scored using a 4-point scale (1=0-25% positive cells; 2=25-50% positive cells; 3=50-75% positive cells; 4=75-100% positive cells). Intensity was scored as absent, weak or strong (1-3, respectively). The two scores were multiplied to yield a final score per pathologist per slide. Inter-observer consistency between pathologists was assessed using Spearman's rank correlation. Statistical significance of ATRX in melanoma progression was assessed using the Kruskal-Wallis one-way analysis of variance test. Significance between any two tissue types was assessed using two-sided Mann-Whitney U tests. This study was conducted according to the Declaration of Helsinki Principles.

cDNA isolation, RT-PCR analysis, and quantitative RT-PCR Analysis. Total RNA from tissues was extracted using RNeasy Kit (Qiagen, Hilden, Germany). A total of 1 ug of RNA was used to synthesize cDNA using the First Strand cDNA synthesis kit (Origene, Rockville, MD). The synthesized cDNA was amplified with primers flanking the ATRX coding regions in five fragments (see Figure 2). PCR reactions were performed using High Fidelity Platinum Taq (Life Technologies, Grand Island, NY) and products were run on a 1% agarose gel and imaged. qPCR analysis for ATRX was conducted as previously described (Kapoor *et al.,* 2010) and

levels were normalized to GAPDH. Approval to collect melanoma specimens was granted by Mount Sinai Biorepository Cooperative and approval to collect benign nevi was granted by Mount Sinai School of Medicine's Division of Dermatopathology (project number 08-0964). The quality of all tissues was assessed by a pathologist and tissues analyzed for PCR had purity of ~95% for benign nevi and ≥80% for metastatic melanoma specimens All primer sequences used for ATRX analysis in this study are listed below:

	Forward Primer (5'-3')	**Reverse Primer (5'-3')**
Fragment 1	ATGACCGCTGAGCCCAT	GGAAGGAACAGACACAATATC
Fragment 2	GAGCACATGCATCAGAATGTTC	GGAATATTTCTCAGTATCAGATGATGAA
Fragment 3	GCTGAGAAGTCAACAGGGAAAG	GCAATCCCACATAAACTGAACAC
Fragment 4	GCAAAGAAAGCAGAGTTGGAAG	GAACCATCTAAACGGTAATAGTCAATG
Fragment 5	GGAAGTGGCAGTGACAATGAT	CATTGATTTCCCTTGGGAAGGT
ATRX N-Term	GTGGTTCTGGAAGTAACTCTGATATG	CGTGACGATCCTGAAGACTTG
ATRX C-Term	GGAGCCCTGTCAGCAATG	GCTGTCACACTGTTTGTTGC
GAPDH	TTTGTCAAGCTCATTTCCTGG	TGATGGTACATGACAAGGTGC