Supplementary Information Titles

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Journal: Nature Medicine

Article Title:	Increased gene copy number of the vesicle SNARE VAMP7 disrupts human male urogenital development through altered estrogen action
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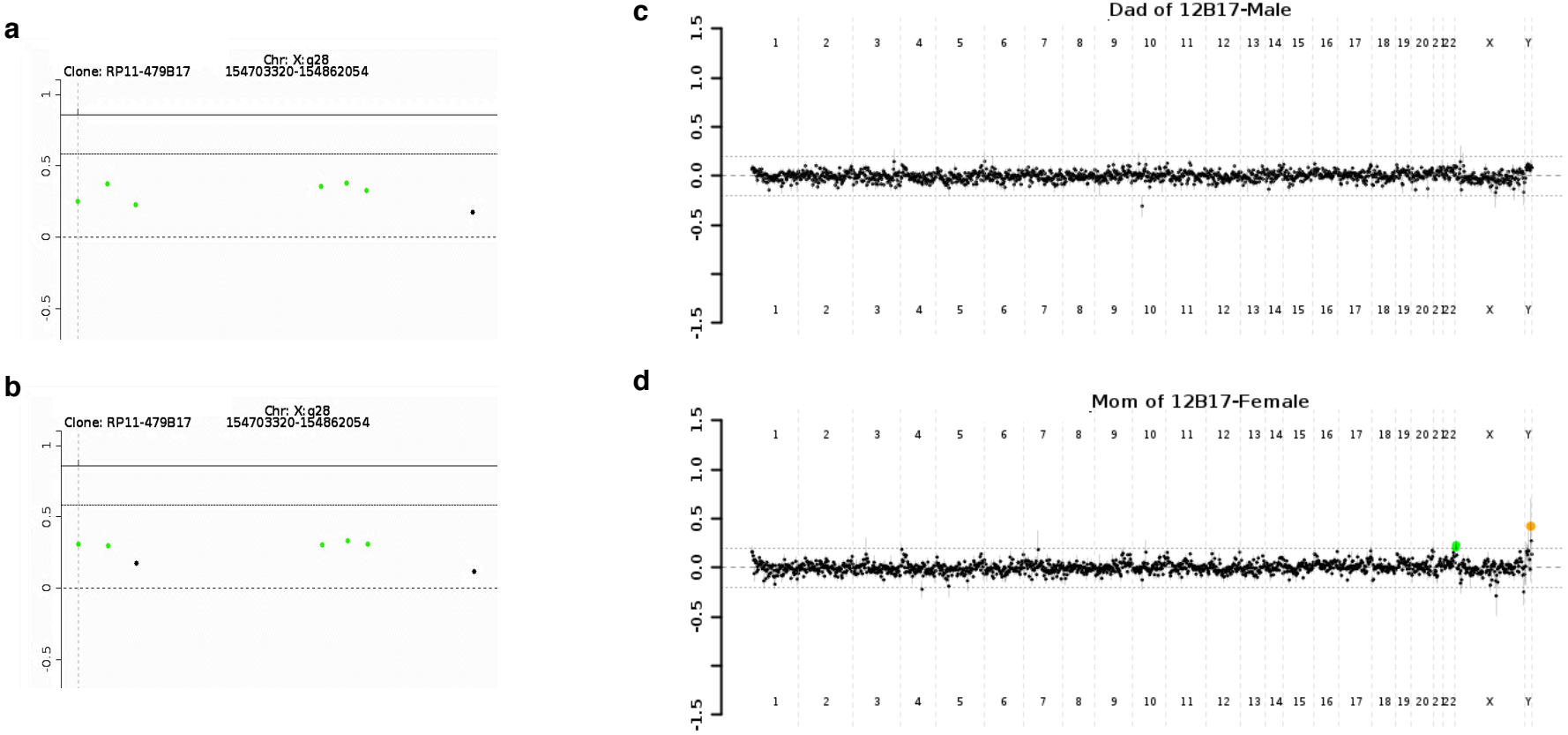
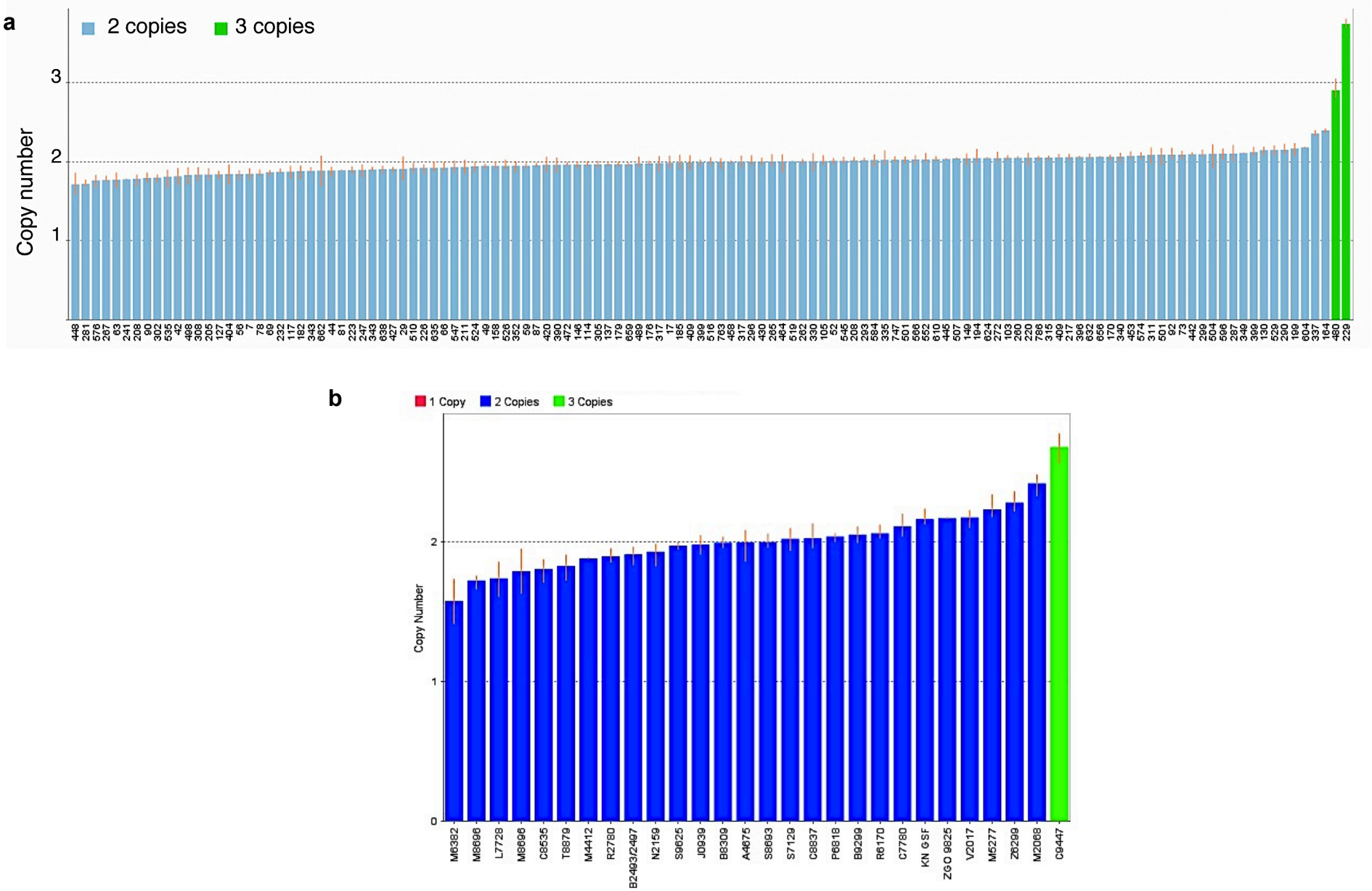
Supplementary Item & Number	Title or Caption
Supplementary Figure S1	**[Genomic hybridization profiles of unrelated 46,XY children presenting with masculinization disorders of the urogenital tract]**
Supplementary Figure S2	[***VAMP7*** **CNV analysis in parental and patients' genomic DNA**]
Supplementary Table 1	[**List of patients with Xq28 duplication encompassing *VAMP7* and presenting with masculinization defects of the genital tract**]
Supplementary Figure S3	[**Impact of VAMP7 on androgen and estrogen receptor signaling**]
Supplementary Figure S4	[**Phenotypic analysis of *VAMP7* BAC transgenic mouse line 21**]



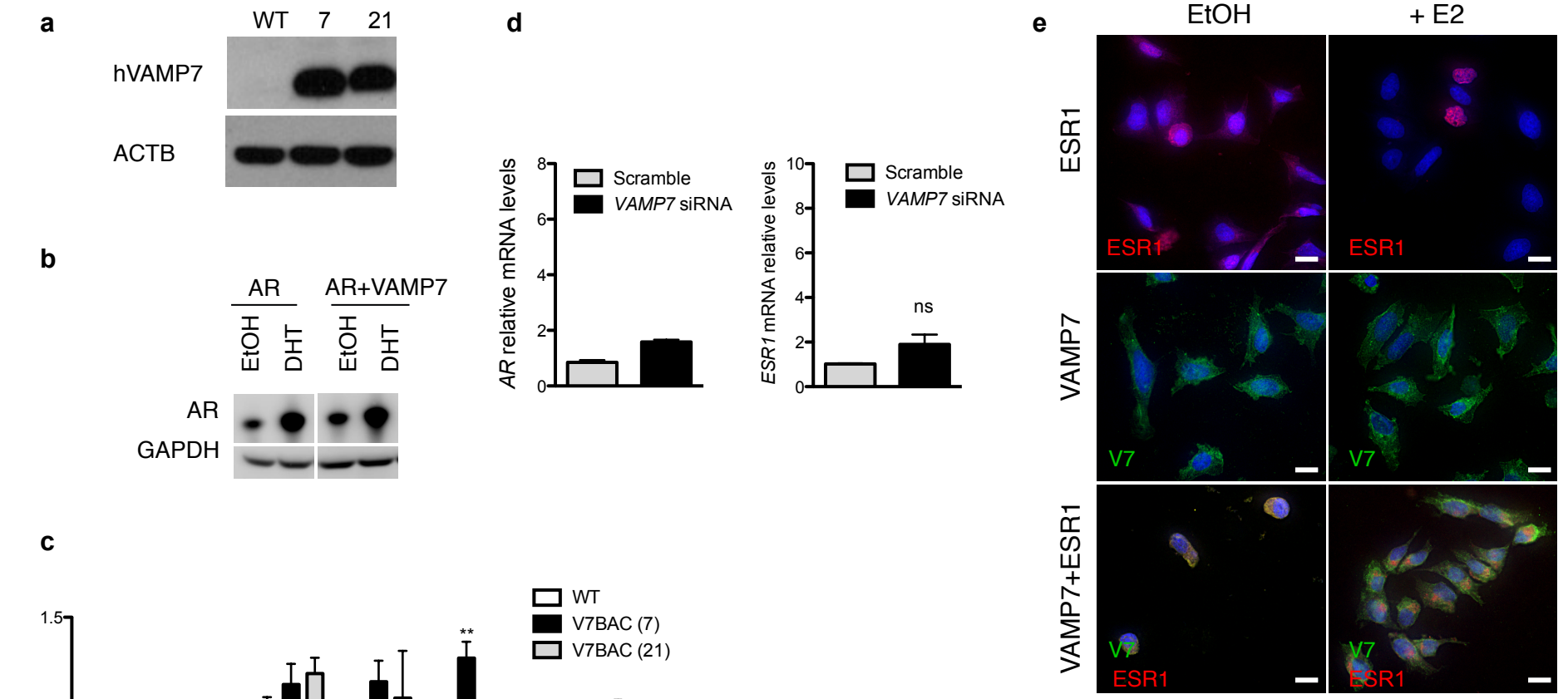
Supplemental Figure S1: Genomic hybridization profiles of unrelated 46,XY children presenting with masculinization disorders of the urogenital tract (**a**,**b**) Enlarged views of genomic hybridization plots showing a positive shift of the probes' signal (green) within a mapped segment of human terminal Xq28 (RP11-479B17), in two unrelated 46,XY subjects presenting with idiopathic hypospadias (12B17; **a**) or cryptorchidism (23B33; **b**). The mean $\log_2$ ratio of intensity of patient *versus* gender-matched reference was shown for each single probe. **(c,d)** Hybridization profiles of genomic DNA from saliva of both unaffected parents of the 46,XY hypospadic child (12B17) harboring a terminal Xq28 gain. The mean $\log_2$ ratio of intensity was presented across individual chromosomes. The colored dots indicate signal shifts of probes, which were not located on Xq28. (**e**) A UCSC genome browser view (http://genome.ucsc.edu/; Human Assembly Mar 2006 (NCBI36/ hg18)) showing RP11-479B17 BAC clone gene coverage (red).



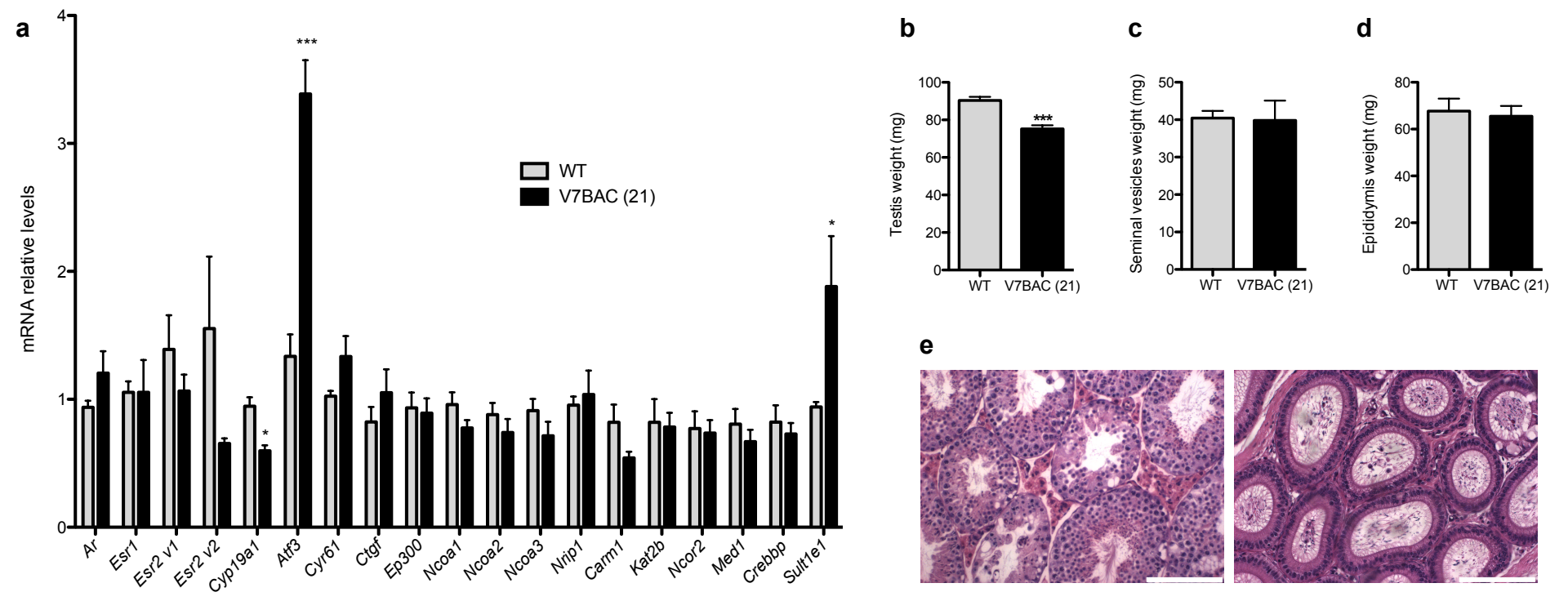
Supplemental Figure S2: CNV Taqman qPCR analysis of *VAMP7* copy number change in distinct cohorts of 46,XY individuals presenting with idiopathic cryptorchidism and/or hypospadias. **(a**) CNV Taqman qPCR analysis of *VAMP7* copy number change in genomic blood DNA from a distinct cohort of unrelated 46,XY subjects (*n* = 180) presenting with cryptorchidism and/or hypospadias. (**b**) CNV Taqman qPCR detection of *VAMP7* copy gain in 28 distinct primary cultures of genital skin fibroblasts from unrelated male newborns presenting with either proximal or mid-shaft hypospadias or cryptorchidism.

Patients	*References*
Patient 1a	Akiyama, M., et al. Functional disomy for Xq26.3-qter in a boy with an unbalanced t(X;21)(q26.3;p11.2) translocation. Am J Med Genet 99 111-114 (2001).
Patient III.4	Vasquez, A.I., Rivera, H., Bobadilla, L. & Crolla, J.A. A familial Xp+ chromosome, dup (Xq26.3-->qter). Journal of medical genetics 32, 891-893 (1995).
Patient III.3	Vasquez, A.I., Rivera, H., Bobadilla, L. & Crolla, J.A. A familial Xp+ chromosome, dup (Xq26.3-->qter). Journal of medical genetics 32, 891-893 (1995).
Case 1	Goodman, B.K., et al. Inherited duplication Xq27-qter at Xp22.3 in severely affected males: molecular cytogenetic evaluation and clinical description in three unrelated families. Am J Med Genet 80, 377-384 (1998).
Patient 3	Lachlan, K.L., et al. Functional disomy resulting from duplications of distal Xq in four unrelated patients. Hum Genet 115, 399-408 (2004).
Patient 1b	Smyk, M., et al. Different-sized duplications of Xq28, including MECP2, in three males with mental retardation, absent or delayed speech, and recurrent infections. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 147B, 799-806 (2008).
Patient 2	Smyk, M., et al. Different-sized duplications of Xq28, including MECP2, in three males with mental retardation, absent or delayed speech, and recurrent infections. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 147B, 799-806 (2008).
Patient 1c	Novelli, A., et al. Disomy of distal Xq in males: case report and overview. Am J Med Genet A 128A, 165-169 (2004).
Patient 1d	Kokalj-Vokac, N., et al. Subterminal deletion/duplication event in an affected male due to maternal X chromosome pericentric inversion. Eur J Pediatr 163, 658-663 (2004).
Patient 1e	Sanlaville, D., et al. Functional disomy of the Xq28 chromosome region. Eur J Hum Genet 13, 579-585 (2005).
Patient 1f	Jezela-Stanek, A., et al. Cryptic x; autosome translocation in a boy--delineation of the phenotype. Pediatric neurology 44, 221-224 (2011).

Supplemental Table 1: List of patients with Xq28 duplication encompassing *VAMP7* and presenting with masculinization defects of the genital tract, based on a literature review. See Figure 1g.



Supplemental Figure S3: Impact of VAMP7 on androgen and estrogen receptor signaling. (**a**) Western blot analysis of human VAMP7 expression in whole testis protein extracts from WT and *V7*BAC transgenic founders (lines 7 and 21). β actin (ACTB) was used as a loading control. (**b**) Western Blot analysis of AR and GAPDH expression in protein extracts of Hela cells co–transfected with AR or with AR and VAMP7, and incubated in absence (EtOH) or presence of dihydrotestosterone (DHT, 10^{-8} M) for 24 h. Samples used for AR and GAPDH stainings derive from the same experiment and the respective gels and blots have been processed in parallel. (**c**) qRT-PCR analysis of endogenous mRNA testicular levels of *Ar* dependent genes normalized to *Gapdh,* in WT and V7BAC mice (lines 7 and 21). Data are presented as mean ± s.e.m. *n* = 3 for each genotype and gene. (**d**) qRT-PCR analysis of endogenous *AR* and *ESR1* mRNA levels normalized to *GAPDH,* in NT2/D1 incubated in presence of scramble or *VAMP7* siRNA. ns: non significant. *n* = 3 independent experiments for each condition. Data are presented as mean ± s.e.m. (**e**) Immunofluorescence staining of VAMP7 and ESR1 in Hela cells following transfection with ESR1, VAMP7 or both, upon stimulation with ethanol (EtOH) or 17 beta estradiol (10^{-8} M) for 24 h. Scale bar, 5 μm.



Supplemental Figure S4: Phenotypic analysis of *VAMP7* BAC transgenic mouse line 21. (**a**) qRT-PCR analysis of key genes involved in estrogen receptor signaling in WT (n = 3 mice) and V7BAC (line 21; n = 3 mice). (**b**) Weights of WT scrotal (n = 8 gonads) and cryptorchid V7BAC testis (n = 6 gonads) from 6-month-old male mice. (**c,d**) Weights of epididymis (**c**) and seminal vesicles (**d**) from 6- month-old male V7BAC (n = 8) and WT (n = 6) mice. (**e**) Hematoxylin- eosin stained images of paraffin-embedded testis and epididymis sections from 6-month-old male V7BAC and WT mice. Scale bar, 125 µm. (**f**) Serum testosterone, 17 beta-estradiol, Lh and Fsh hormone levels in 6- month-old male V7BAC transgenic and WT mice. **(g)** Epididymal sperm count and motility in 6- month-old WT animals (n = 6) and *V7*BAC mice (n = 5). **(h)** Litter size from WT (n = 12 litters) and V7BAC (n = 24 litters) mice in a 4 month -continuous mating study. All data are expressed as mean ± s.e.m. Statistical significance was determined by unpaired, two-tailed Student's t-test. *P < 0.05; **P < 0.01; ***P < 0.001.