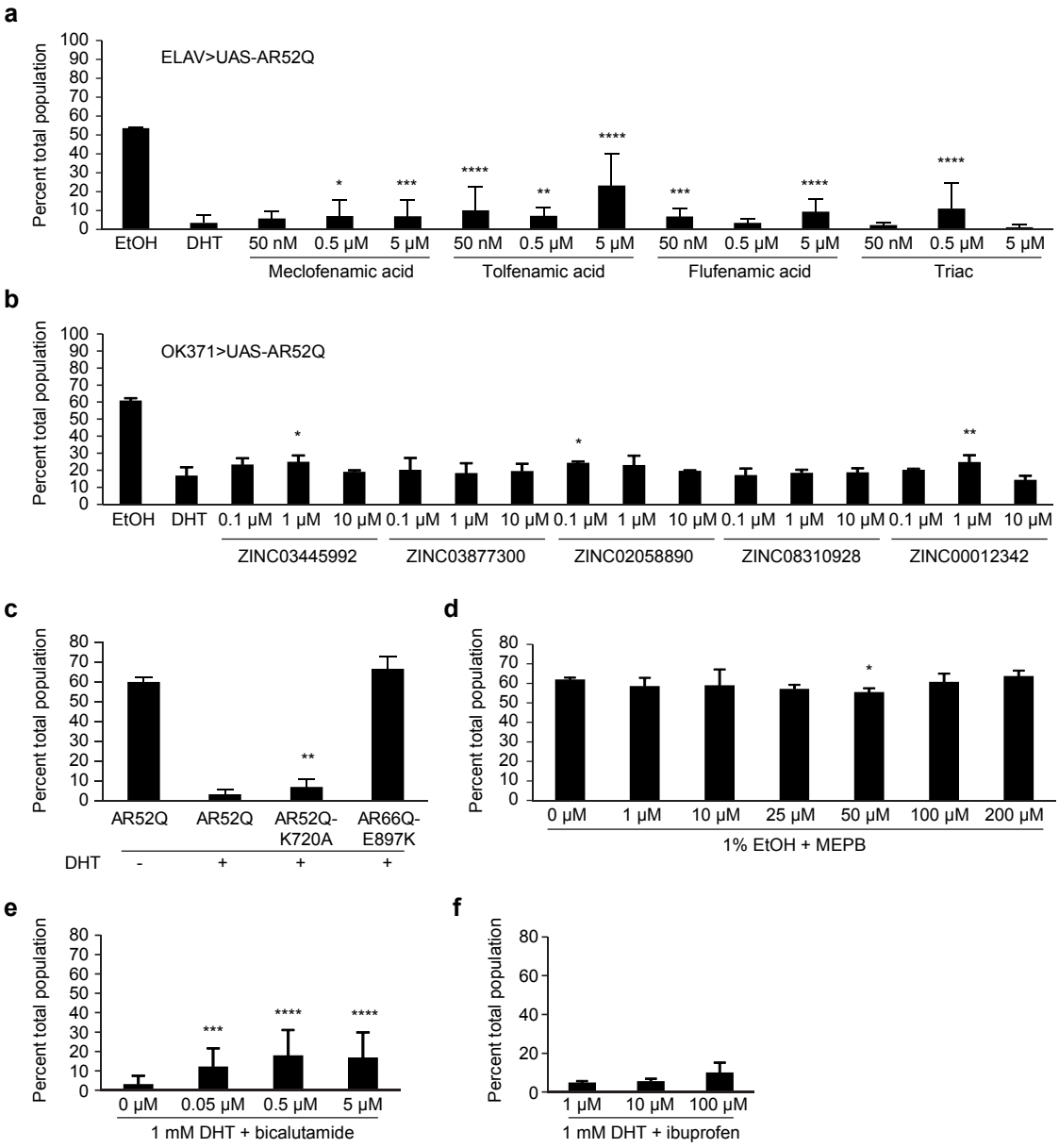

Supplementary Figure 1. Screening of AF2-modulating compounds in a *Drosophila* model of SBMA.

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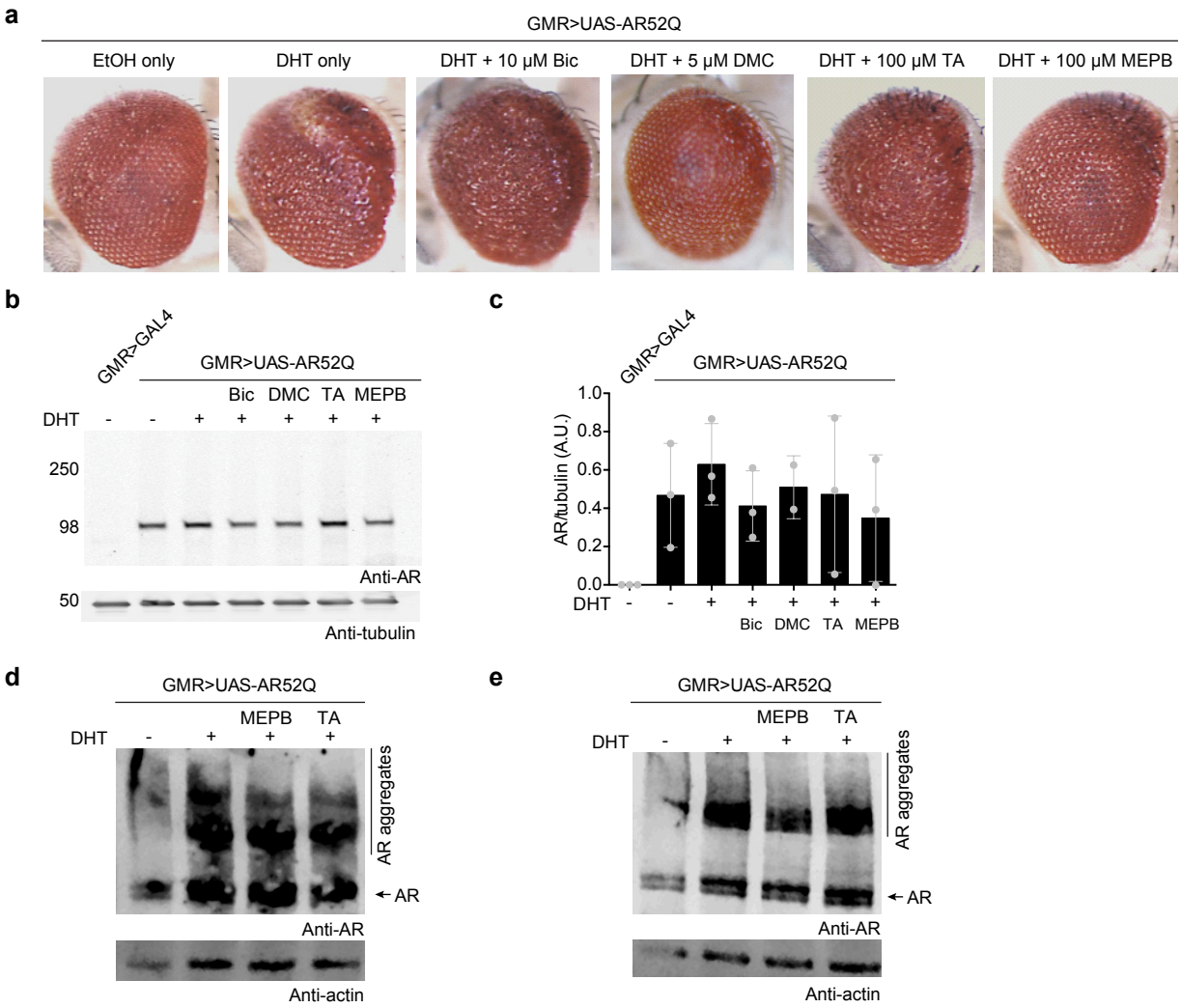
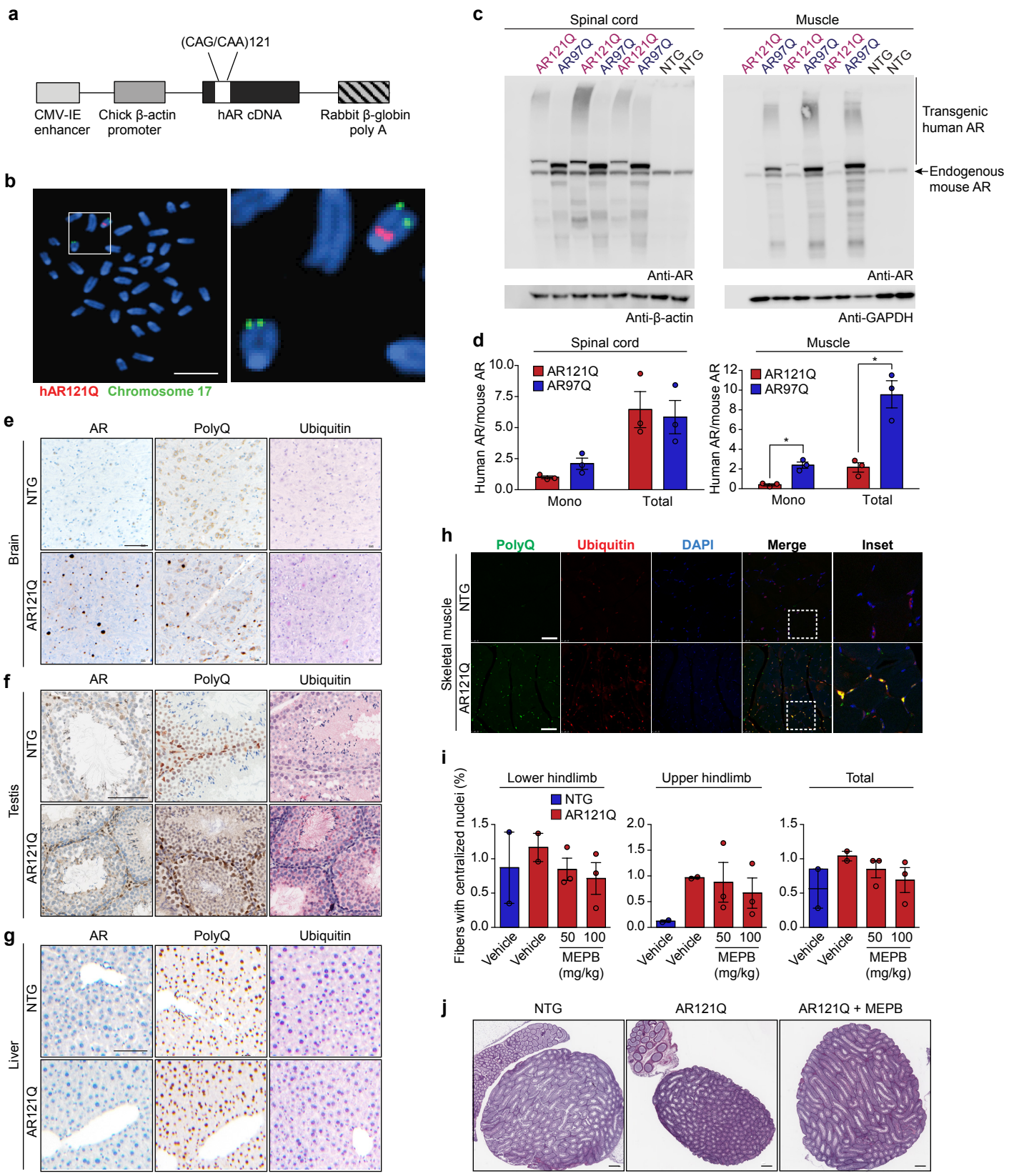
Supplementary Figure 1. Screening of AF2-modulating compounds in a *Drosophila* model of SBMA. (**a**) Mean viability of SBMA flies (AR52Q) reared on food containing vehicle (EtOH), DHT, or DHT in addition to the AF2 modulators described by Estebanez-Perpina et al. (2007)[19]. *P = 0.03376 for 0.5 μM Meclofenamic acid, ***P = 0.000228 for 5 μM Meclofenamic acid, ****P ≤ 0.0001 for 50 nM and 5 μM Tolfenamic acid, **P = 0.006852 for 0.5 μM Tolfenamic acid, ***P = 0.000314 for 50 nM Flufenamic acid, ****P ≤ 0.0001 for 5 μM Flufenamic acid, ****P ≤ 0.0001 for 0.5 μM Triac. (**b**) Mean viability of SBMA flies reared on food containing vehicle, DHT, or DHT in addition to the AF2 modulators described by Lack et al. (2011)[21]. *P = 0.030346 for 1 μM ZINC03445992, *P = 0.020803 for 0.1 μM ZINC02058890, **P = 0.008581 for 1 μM ZINC00012342. (**c**) Mean viability of transgenic flies expressing AR52Q (ELAV>UAS-AR52Q) reared on food containing vehicle or DHT and transgenic flies expressing AR52Q-K720A or AR66Q-E897K reared on food containing DHT. **P = 0.001136 for AR52Q-K720A. (**d**) Mean viability of SBMA flies (ELAV>UAS-AR52Q) reared on food containing MEPB in the absence of DHT. *P = 0.023999 for 50 μM MEPB. (**e**) Mean viability of SBMA flies (ELAV>UAS-AR52Q) reared on food containing DHT and bicalutamide. ***P = 0.00081 for 0.05 μM Bicalutamide, ****P ≤ 0.0001 for 0.5 μM and 5 μM Bicalutamide. (**f**) Mean viability of SBMA flies (ELAV>UAS-AR52Q) reared on food containing DHT and ibuprofen. All data were evaluated by Chi-square analysis; n = 50 adult flies/treatment group for all experiments. Comparisons of data shown in **a**, **b**, **c**, and **e** were made between the actual population frequencies of each treatment group and the predicted population frequency determined by the sum of all treatment groups of AR52Q flies. In **d**, the predicted population frequency of each treatment group was determined by the ethanol + DMSO group. In **f**, the predicted population frequency of each treatment group was determined by the DHT + 1 μM ibuprofen group. All graphs represent mean ± s.e.m.



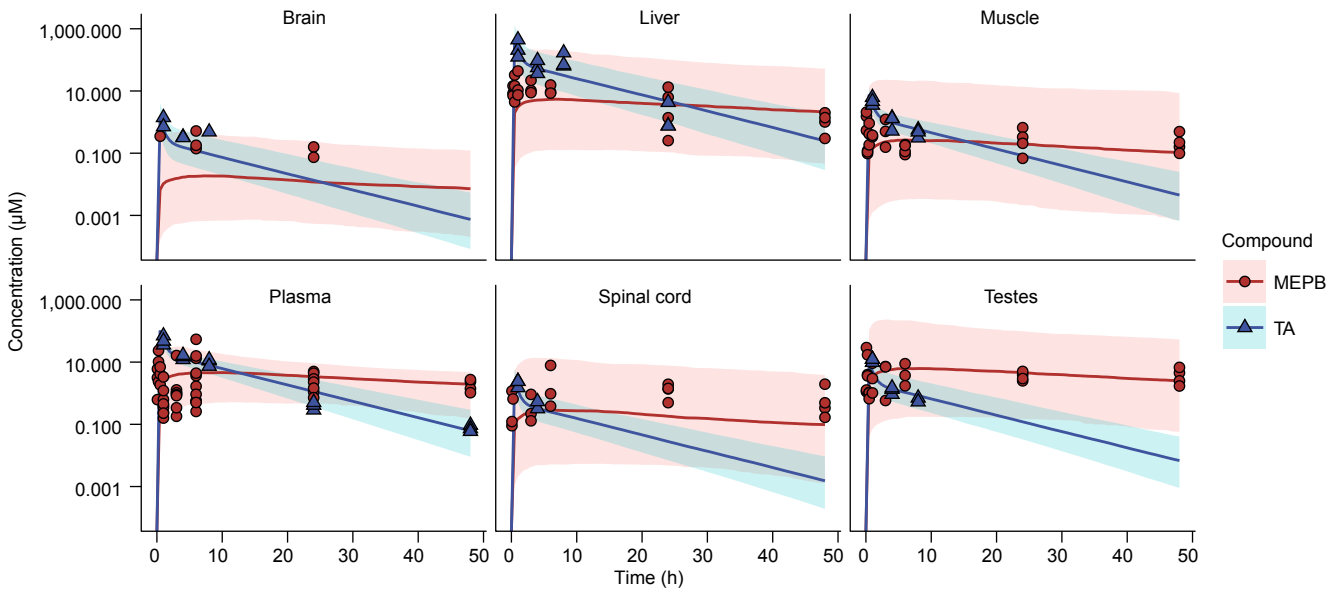
Supplementary Figure 2. AF2 modulation rescues SBMA fly eye degeneration without reducing AR levels. (**a**) Representative images of SBMA fly eyes reared on food containing vehicle (EtOH), DHT, or DHT + bicalutamide (Bic), dimethylcurcumin (DMC), TA, or MEPB. Images were captured from four flies for each treatment group. (**b**) Representative immunoblot of AR expression in SBMA fly heads. (**c**) Quantification of protein levels from three immunoblots, as depicted in **b**. Graph represents mean ± s.e.m.(**d**) Flies expressing AR52Q in eyes were fed with drugs in their adult stage for 4 days. Representative WB image from three independent experiments is shown. (**e**) Flies expressing AR52Q were raised on food containing drugs during their development from their embryonic stage. Eclosed adults were immediately collected and processed. Three heads for each treatment were used to detect aggregation. Representative WB image from three independent experiments is shown.



Supplementary Figure 3. Generation and characterization of a novel mouse model of SBMA. *Continued on next page.*

Supplementary Figure 3 continued. Generation and characterization of a novel mouse model of SBMA. (**a**) Schematic depicting the cDNA construct used to generate transgenic SBMA mice. (**b**) Fluorescence in situ hybridization of an AR121Q-specific probe (red) and a general marker of chromosome 17 (green) in chromosome spreads isolated from lungs of AR121Q mice. Representative image from two indenpendent experiments. Scale bar, 10 μm. (**c**) Western blot analysis of spinal cord and muscle expression of transgenic human AR in AR121Q mice and a previously published SBMA mouse model (AR97Q). (**d**) Quantification of the levels of transgenic human AR compared with that of endogenous mouse AR in AR121Q and AR97Q mice. n = 3 mice per group, **P* = 0.00396968 for mono and **P* = 0.00703522 for total by unpaired multiple t-test, two-tailed. (**e-g**) Representative brain (**e**), testis (**f**) and liver (**g**) sections from 7-week-old NTG and AR121Q mice from one independent experiment. Sections were stained with H&E for assessment of morphology, in addition to AR (N20) and ubiquitin antibodies. Scale bars represent 100 μm. (**h**) PolyQ and ubiquitin costaining in skeletal muscle of NTG and AR121Q mice. Representative images are shown from one independent experiment. (**i**) Quantification of fibers with centralized nuclei in lower and upper hindlimb muscles. n = 2, 2, 3, and 3 mice for NTG, vehicle, 50 mg/kg, and 100 mg/kg MEPB treated AR121Q groups. Graphs represent mean ± s.e.m. (**j**) Representative images of testis from NTG, AR121Q and AR121Q treated with 100 mg/kg MEPB mice from one independent experiment. Scale bars represent 500 μm. All graphs represent mean ± s.e.m.

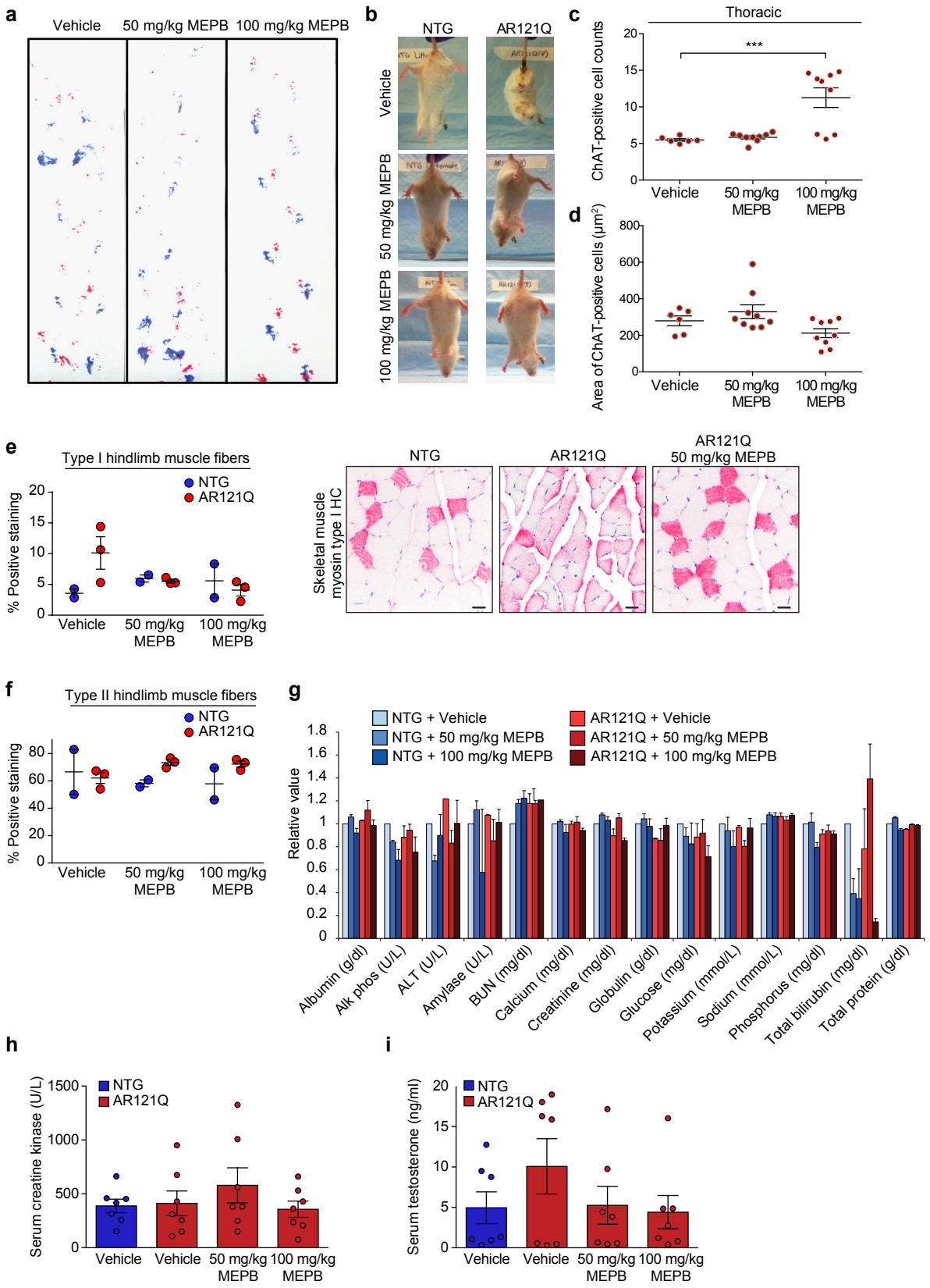
a



b

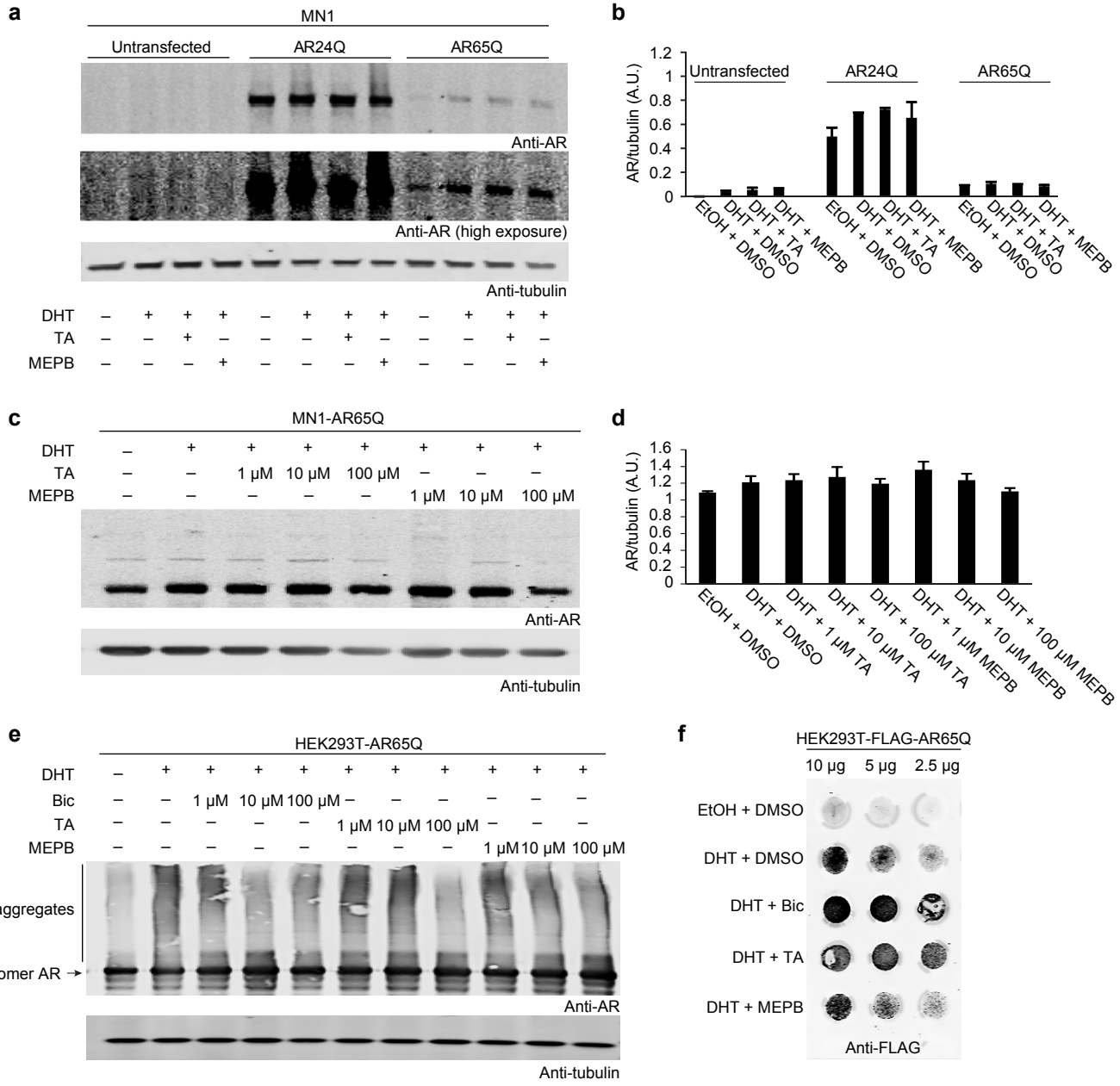
Compound	Tissue	Cmax (µM)	Tmax (h)	AUClast (µM*h)	T1/2 (h)	AUCinf (µM*h)
MEPB	Plasma	5.06 [0.563, 33.5]	4.56 [0.960, 32.2]	184 [24.0, 497]	45.2 [6.11, 400]	379 [199, 787]
TA	Plasma	97.6 [81.9, 115]	0.480 [0.480, 0.480]	229 [142, 345]	5.70 [4.50, 7.41]	230 [142, 348]
MEPB	Liver	6.07 [0.158, 263]	4.56 [0.960, 32.2]	190 [5.77, 5910]	45.2 [6.11, 400]	515 [21.7, 9920]
TA	Liver	403 [125, 1290]	0.480 [0.480, 0.480]	934 [285, 3220]	5.70 [4.50, 7.41]	936 [286, 3220]
MEPB	Muscle	0.315 [0.00399, 27.4]	4.56 [0.960, 32.2]	10.2 [0.124, 704]	45.1 [6.11, 394]	23.0 [0.423, 1600]
TA	Muscle	7.24 [4.45, 12.4]	0.480 [0.480, 0.480]	17.1 [8.97, 33.3]	5.70 [4.50, 7.41]	17.1 [8.98, 33.6]
MEPB	Testes	7.22 [0.209, 259]	4.56 [0.960, 32.2]	236 [8.65, 5340]	45.2 [6.11, 400]	551 [33.5, 11000]
TA	Testes	10.4 [4.25, 28.7]	0.480 [0.480, 0.480]	24.5 [9.58, 72.4]	5.70 [4.50, 7.41]	24.6 [9.58, 72.7]
MEPB	Brain	0.0216 [7.68E-04, 0.547]	4.56 [0.960, 32.2]	0.677 [0.0314, 12.3]	45.2 [6.11, 400]	1.73 [0.129, 24.8]
TA	Brain	1.18 [0.362, 4.00]	0.480 [0.480, 0.480]	2.76 [0.799, 9.49]	5.70 [4.50, 7.41]	2.77 [0.801, 9.51]
MEPB	Spinal cord	0.334 [0.00594, 15.6]	4.56 [0.960, 32.2]	10.1 [0.235, 383]	45.2 [6.11, 401]	22.0 [0.958, 767]
TA	Spinal cord	2.46 [1.02, 6.05]	0.480 [0.480, 0.480]	5.73 [2.22, 15.3]	5.70 [4.50, 7.41]	5.74 [2.22, 15.4]

Supplementary Figure 4. Pharmacokinetics of MEPB and TA. (**a**) MEPB and TA concentrations in brain, liver, muscle, plasma, spinal cord, and testes were measured at multiple time points (1–48 h) following a single intraperitoneal injection of MEPB 100 mg/kg or TA 50 mg/kg in NTG mice. Each solid circle or triangle indicates one observed concentration or sample. The solid line represents the median PK model predicted Ct profile from Monte Carlo simulations, with the shaded area representing the 90% model prediction interval. (**b**) Summary of NCA PK parameter estimates from model-driven Monte Carlo simulations (n = 1000). Parameters are reported as the median and the 90% prediction interval.

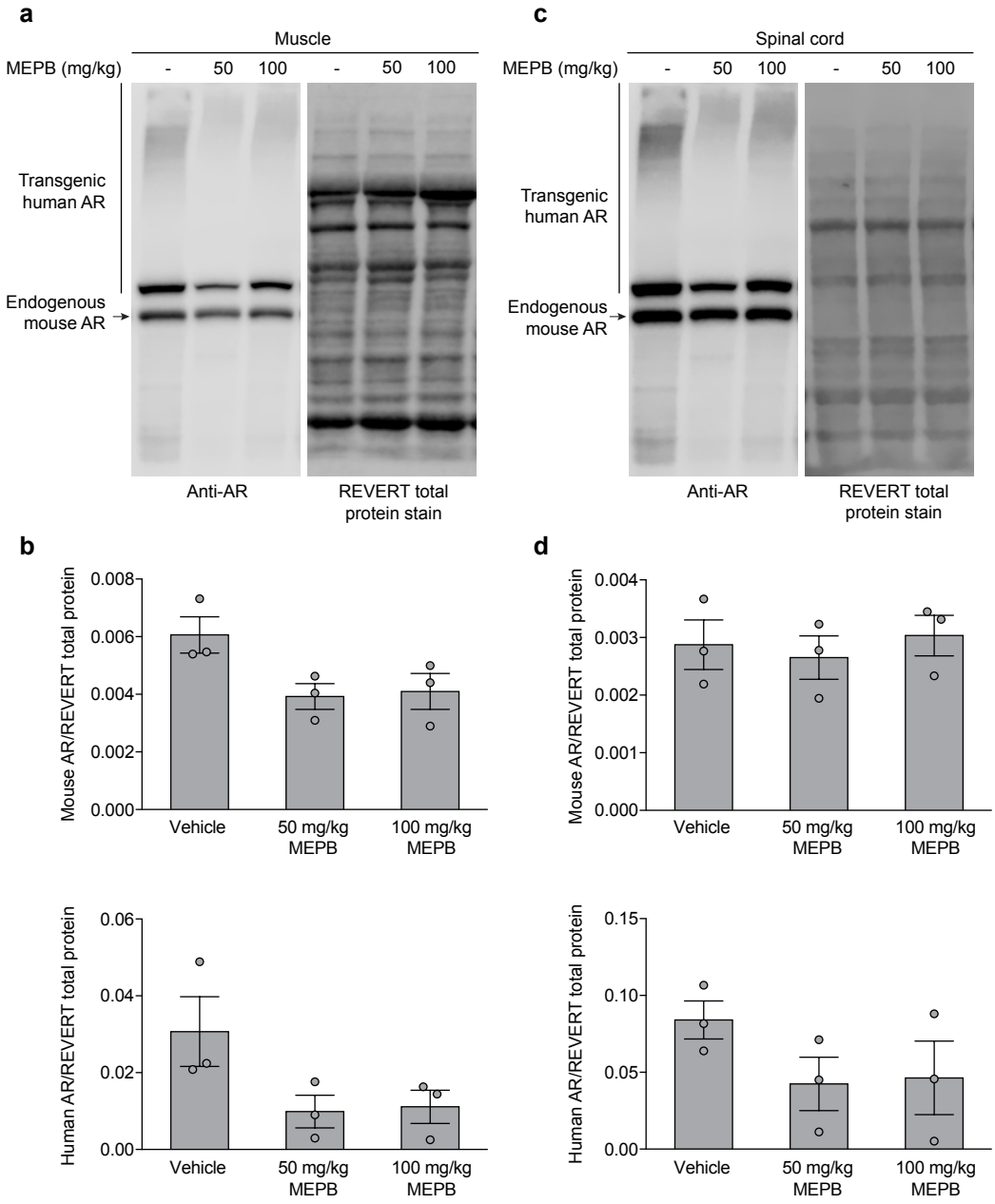


Supplementary Figure 5. Effect of MEPB treatment on gait, clasping phenotype, muscle fiber type, and blood chemistry. *Continued on next page.*

Supplementary Figure 5 continued. Effect of MEPB treatment on gait, clasping phenotype, muscle fiber type, and blood chemistry. (**a**) Representative footprint/gait analysis of 8-week-old AR121Q mice treated with MEPB from three independent experiments. Hindpaws were painted blue and forepaws were painted red. (**b**) Video stills of clasping behavior in representative NTG and AR121Q mice from three independent experiments. (**c, d**) Quantification of the number (**c**) and size (**d**) of ChAT-positive motor neurons in the anterior horn of the thoracic spinal cord of AR121Q mice treated with vehicle, 50 mg/kg MEPB, or 100 mg/kg MEPB. Data were evaluated by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test, n = 6, 9, and 9 slides (c) and sections (d) for vehicle, 50 mg/kg, and 100 mg/kg MEPB treated AR121Q groups. (**e**) Graph (left) showing quantification of type I hindlimb muscle fibers in NTG and AR121Q mice. Classification of type I myofibers was determined by positive staining for the type I MHC antibody. Representative histology images (right) are shown for NTG, untreated AR121Q mice, and treated AR121Q mice. Scale bar, 20 μm. (**f**) Quantification of type II hindlimb muscle fiber staining in NTG and AR121Q mice. Classification of type II myofibers was determined by positive staining for the type II (IIa and IIb) MHC antibody, n = 2 (NTG) and 3 (AR121Q) mice per treatment group. (**g**) Quantification of mean blood concentrations of metabolites, electrolytes, enzymes, and other molecules associated with kidney and liver function from NTG (blue bars) and AR121Q (red bars) mice. Mean concentrations were normalized to vehicle-treated NTG mice to allow depiction of all chemistries on one graph, n = 2 mice per treatment group. (**h,i**) Quantification of mean blood concentrations of serum creatine kinase (**h**) and serum testosterone (**i**) in NTG and AR121Q mice treated with vehicle, 50 mg/kg MEPB, or 100 mg/kg MEPB; n = 7 mice per treatment group. Data were evaluated by ordinary one-way ANOVA. All graphs represent mean ± s.e.m.



Supplementary Figure 6. Effect of AF2 modulators on AR protein levels in MN1 and HEK293T cells. (**a**) Representative immunoblot of MN1 cells that were untransfected, stably transfected with AR24Q, or stably transfected with AR65Q. Cells were treated with vehicle (0.1% ethanol + 0.1% DMSO), 10 nM DHT + 0.1% DMSO, 10 nM DHT + 10 μM TA, or 10 nM DHT + 10 μM MEPB for 24 h. Blots were stained with AR (D6F11) and tubulin antibodies. (**b**) Quantification of AR protein levels from three immunoblots, as depicted in **a**. (**c**) Representative immunoblot of stably transfected MN1-AR65Q cells treated with 10 nM DHT + TA or MEPB. (**d**) Quantification of AR protein levels from three immunoblots, as depicted in **c**. (**e**) Representative immunoblot of HEK293T cells transiently transfected with AR65Q for 24 h. Cells were treated with vehicle, 10 nM DHT, or 10 nM DHT + bicalutamide (Bic), TA, or MEPB for 24 h, and blots (n = 3) were stained with AR (D6F11) and tubulin antibodies. (**f**) Filter trap assay for AR aggregates from lysates prepared from HEK293T cells transiently transfected with FLAG-AR65Q. Cellulose acetate membranes (n = 3) were stained with FLAG (M2) antibody. All graphs represent mean ± s.e.m.



Supplementary Figure 7. Effect of AF2 modulators on AR protein levels in AR121Q mice. (**a,c**) Representative immunoblot of skeletal muscle (**a**) and spinal cord (**c**) of AR121Q mice treated with vehicle, 50 mg/kg MEPB, or 100 mg/kg MEPB. (**b,d**) Quantification of mouse AR and human AR protein levels relative to REVERT total protein stain, n = 3 mice per treatment group. Data were analyzed by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test compared to vehicle. All graphs represent mean ± s.e.m.

Assay	Type I error rate	Power	Detection difference	Minimum number of mice/group required
Body weight	0.05	0.8	4 g	4
Rotarod	0.05	0.8	50 s	5
Grip strength	0.05	0.8	20 g	10
Survival	0.05	0.8	47%	10

Supplementary Table 1. Statistical variables used to determine sample size.