Table S1

siRNA	sense (5'-3')	antisense (5'-3')
asiAR-001	GAGAUGAAGCUUCUGG	CCAGAAGCUUCAUCUCCACAG
asiAR-002	GGAGAUGAAGCUUCUG	CAGAAGCUUCAUCUCCACAGA
asiAR-003	GUGGAGAUGAAGCUUC	GAAGCUUCAUCUCCACAGAUC
asiAR-004	UGUGGAGAUGAAGCUU	AAGCUUCAUCUCCACAGAUCA
asiAR-005	UCUGUGGAGAUGAAGC	GCUUCAUCUCCACAGAUCAGG
asiAR-006	UGAUCUGUGGAGAUGA	UCAUCUCCACAGAUCAGGCAG
asiAR-007	CUGAUCUGUGGAGAUG	CAUCUCCACAGAUCAGGCAGG
asiAR-008	AAGACCUGCCUGAUCU	AGAUCAGGCAGGUCUUCUGGG
asiAR-009	UUUCCACCCCAGAAGA	UCUUCUGGGGUGGAAAGUAAU
asiAR-010	ACUUUCCACCCCAGAA	UUCUGGGGUGGAAAGUAAUAG
asiAR-011	AAGGGAAACAGAAGUA	UACUUCUGUUUCCCUUCAGCG
asiAR-012	GAAGGGAAACAGAAGU	ACUUCUGUUUCCCUUCAGCGG
asiAR-013	CUGAAGGGAAACAGAA	UUCUGUUUCCCUUCAGCGGCU
asiAR-014	CAAAAGAGCCGCUGAA	UUCAGCGGCUCUUUUGAAGAA
asiAR-015	UCAAAAGAGCCGCUGA	UCAGCGGCUCUUUUGAAGAAG
asiAR-016	CUUCAAAAGAGCCGCU	AGCGGCUCUUUUGAAGAAGAC
asiAR-017	CUUCUUCAAAAGAGCC	GGCUCUUUUGAAGAAGACCUU
asiAR-018	UCUUCUUCAAAAGAGC	GCUCUUUUGAAGAAGACCUUG
asiAR-019	AGGUCUUCUUCAAAAG	CUUUUGAAGAAGACCUUGCAG
asiAR-020	AAGCAGGGAUGACUCU	AGAGUCAUCCCUGCUUCAUAA
asiAR-021	UGAAGCAGGGAUGACU	AGUCAUCCCUGCUUCAUAACA
asiAR-022	UUAUGAAGCAGGGAUG	CAUCCCUGCUUCAUAACAUUU
asiAR-023	UGUUAUGAAGCAGGGA	UCCCUGCUUCAUAACAUUUCC
asiAR-024	AUGUUAUGAAGCAGGG	CCCUGCUUCAUAACAUUUCCG
asiAR-025	GCUAUGAAUGUCAGCC	GGCUGACAUUCAUAGCCUUCA
asiAR-026	GGCUAUGAAUGUCAGC	GCUGACAUUCAUAGCCUUCAA
asiAR-027	GAAGGCUAUGAAUGUC	GACAUUCAUAGCCUUCAAUGU
asiAR-028	UUGAAGGCUAUGAAUG	CAUUCAUAGCCUUCAAUGUGU
asiAR-029	GAAGCCAUUGAGCCAG	CUGGCUCAAUGGCUUCCAGGA
asiAR-030	CUGGCUUCCGCAACUU	AAGUUGCGGAAGCCAGGCAAG
asiAR-031	UGCCUGGCUUCCGCAA	UUGCGGAAGCCAGGCAAGGCC
asiAR-032	AGUGGGCCAAGGCCUU	AAGGCCUUGGCCCACUUGACC
asiAR-033	CCAGGAUGCUCUACUU	AAGUAGAGCAUCCUGGAGUUG
asiAR-034	UCCAGGAUGCUCUACU	AGUAGAGCAUCCUGGAGUUGA
asiAR-035	AACUCCAGGAUGCUCU	AGAGCAUCCUGGAGUUGACAU
asiAR-036	UACCGCAUGCACAAGU	ACUUGUGCAUGCGGUACUCAU
asiAR-037	AGUACCGCAUGCACAA	UUGUGCAUGCGGUACUCAUUG
asiAR-038	CAAUGAGUACCGCAUG	CAUGCGGUACUCAUUGAAAAC
asiAR-039	UCAAUGAGUACCGCAU	AUGCGGUACUCAUUGAAAACC
asiAR-040	UUCAAUGAGUACCGCA	UGCGGUACUCAUUGAAAACCA
asiAR-041	UUGGAUGGCUCCAAAU	AUUUGGAGCCAUCCAAACUCU
asiAR-042	AGUUUGGAUGGCUCCA	UGGAGCCAUCCAAACUCUUGA
asiAR-043	AGAGUUUGGAUGGCUC	GAGCCAUCCAAACUCUUGAGA
asiAR-044	UCAAGGAACUCGAUCG	CGAUCGAGUUCCUUGAUGUAG
asiAR-045	CAUCAAGGAACUCGAU	AUCGAGUUCCUUGAUGUAGUU
asiAR-046	CUACAUCAAGGAACUC	GAGUUCCUUGAUGUAGUUCAU
asiAR-047	GAACUACAUCAAGGAA	UUCCUUGAUGUAGUUCAUUCG
asiAR-048	CUUCGAAUGAACUACA	UGUAGUUCAUUCGAAGUUCAU
asiAR-049	UGAACUUCGAAUGAAC	GUUCAUUCGAAGUUCAUCAAA
asiAR-050	UGAUGAACUUCGAAUG	CAUUCGAAGUUCAUCAAAGAA
asiAR-051	GGGCUGAAAAAUCAAA	UUUGAUUUUUCAGCCCAUCCA
asiAR-052	GAUGGGCUGAAAAAUC	GAUUUUUCAGCCCAUCCACUG
asiAR-053	UAUUCCAGUGGAUGGG	CCCAUCCACUGGAAUAAUGCU
asiAR-054	CAUUAUUCCAGUGGAU	AUCCACUGGAAUAAUGCUGAA
asiAR-055	AGCAUUAUUCCAGUGG	CCACUGGAAUAAUGCUGAAGA
asiAR-056	UUCAGCAUUAUUCCAG	CUGGAAUAAUGCUGAAGAGAG
asiAR-057	CUCUUCAGCAUUAUUC	GAAUAAUGCUGAAGAGAGCAG
asiAR-058	CUGCUCUCUUCAGCAU	AUGCUGAAGAGAGCAGUGCUU
asiAR-059	AAGCACUGCUCUCUUC	GAAGAGAGCAGUGCUUUCAUG
asiAR-060	GAAAGCACUGCUCUCU	AGAGAGCAGUGCUUUCAUGCA
asiAR-061	CAUGAAAGCACUGCUC	GAGCAGUGCUUUCAUGCACAG
asiAR-062	GUGCAUGAAAGCACUG	CAGUGCUUUCAUGCACAGGAA
asiAR-063	UUCCUGUGCAUGAAAG	CUUUCAUGCACAGGAAUUCCU
asiAR-064	GAAUUCCUGUGCAUGA	UCAUGCACAGGAAUUCCUGGG
asiAR-065	AGGAAUUCCUGUGCAU	AUGCACAGGAAUUCCUGGGGG
asiAR-066	UCACCAAGCUCCUGGA	UCCAGGAGCUUGGUGAGCUGG
asiAR-067	ACCAGCUCACCAAGCU	AGCUUGGUGAGCUGGUAGAAG
asiAR-068	CUACCAGCUCACCAAG	CUUGGUGAGCUGGUAGAAGCG
asiAR-069	ACCUGCUAAUCAAGUC	GACUUGAUUAGCAGGUCAAAA
asiAR-070	GACCUGCUAAUCAAGU	ACUUGAUUAGCAGGUCAAAAG
asiAR-071	UUUGACCUGCUAAUCA	UGAUUAGCAGGUCAAAAGUGA
asiAR-072	CUUUUGACCUGCUAAU	AUUAGCAGGUCAAAAGUGAAC
asiAR-073	UCACUUUUGACCUGCU	AGCAGGUCAAAAGUGAACUGA
asiAR-074	UUCACUUUUGACCUGC	GCAGGUCAAAAGUGAACUGAU
asiAR-075	CAGUUCACUUUUGACC	GGUCAAAAGUGAACUGAUGCA
asiAR-076	CAUCAGUUCACUUUUG	CAAAAGUGAACUGAUGCAGCU
asiAR-077	CUGCAUCAGUUCACUU	AAGUGAACUGAUGCAGCUCUC
asiAR-078	GCUGCAUCAGUUCACU	AGUGAACUGAUGCAGCUCUCU
asiAR-079	CCAUCUAUUUCCACAC	GUGUGGAAAUAGAUGGGCUUG
asiAR-080	CCCAUCUAUUUCCACA	UGUGGAAAUAGAUGGGCUUGA
asiAR-081	AGCCCAUCUAUUUCCA	UGGAAAUAGAUGGGCUUGACU
asiAR-082	UCAAGCCCAUCUAUUU	AAAUAGAUGGGCUUGACUUUC
asiAR-083	GGAAAGUCAAGCCCAU	AUGGGCUUGACUUUCCCAGAA
asiAR-084	CUGGGAAAGUCAAGCC	GGCUUGACUUUCCCAGAAAGG
asiAR-085	UUUCUGGGAAAGUCAA	UUGACUUUCCCAGAAAGGAUC
asiAR-086	UCCUUUCUGGGAAAGU	ACUUUCCCAGAAAGGAUCUUG
asiAR-087	CCAAGAUCCUUUCUGG	CCAGAAAGGAUCUUGGGCACU
asiAR-088	UGCCCAAGAUCCUUUC	GAAAGGAUCUUGGGCACUUGC

Table S1. List of 88 asiRNA targeting AR

Fig S1

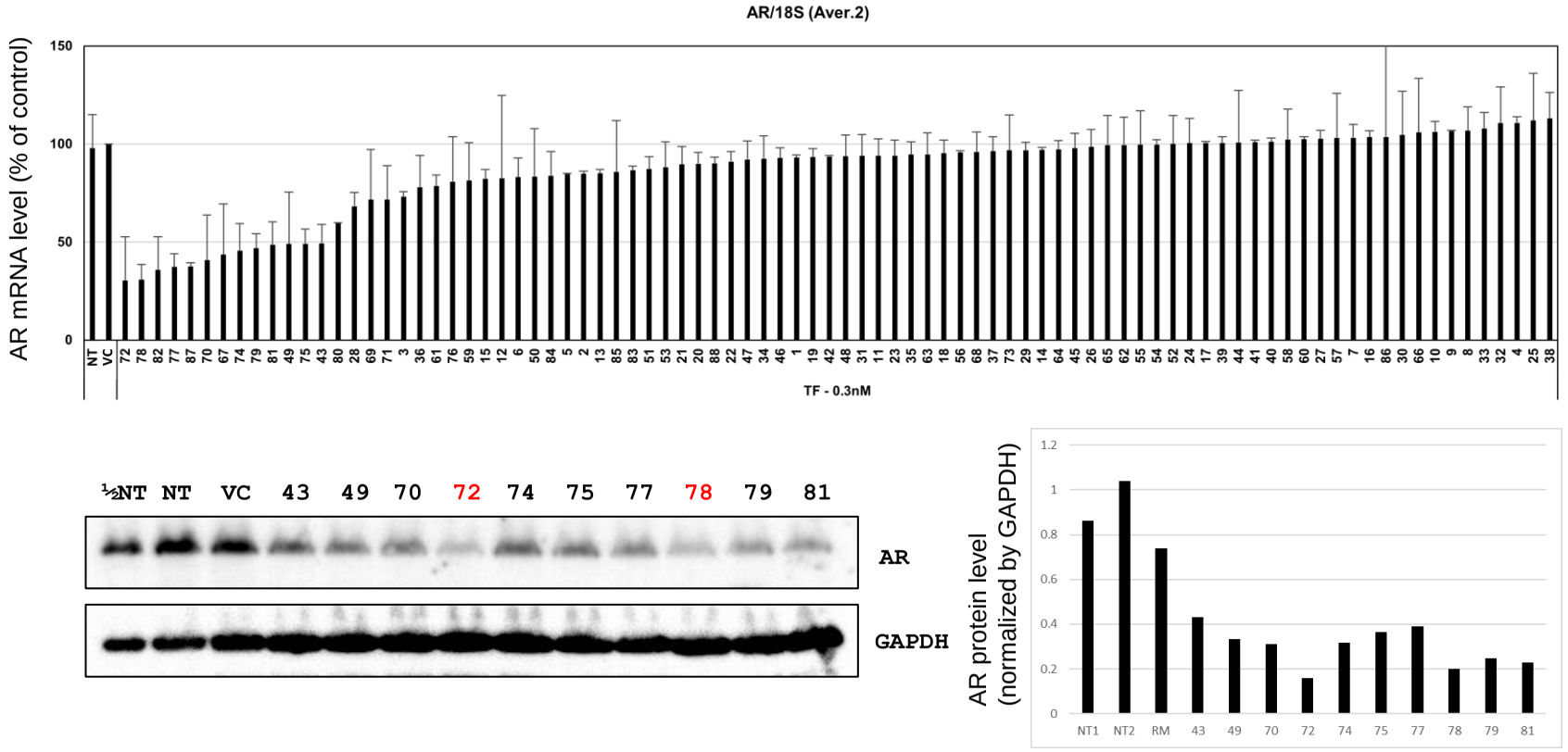
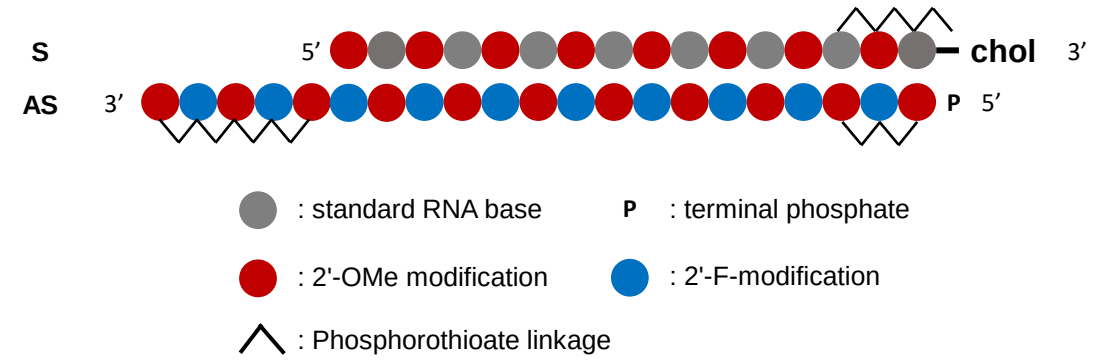


Fig S1. KD efficacy of 88 asiAR in *in vitro* screening
(Top) A549 cells were transfected with 0.3 nM asiRNA targeting AR using RNAiMAX as a transfection agent. NT: non-treated, VC: RNAiMAX only. Relative AR mRNA level normalized by 18S RNA was shown (n=2). AR and GAPDH protein level was shown by immunoblotting in selected samples. (Bottom) AR protein level was quantitated by measuring relative intensity of AR over GAPDH protein band.

Fig S2



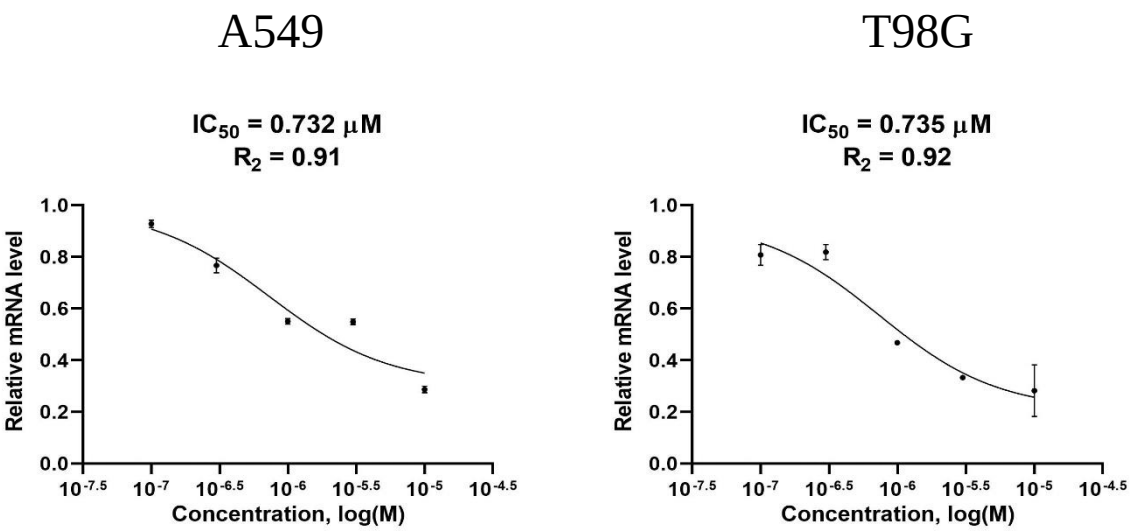


Fig S2. (Top) Structure of cp-asiAR. (Bottom) mRNA knockdown data was used to calculate IC50 value. Graph was drawn using from the Prism 8.0 software (GraphPad).

Fig S3

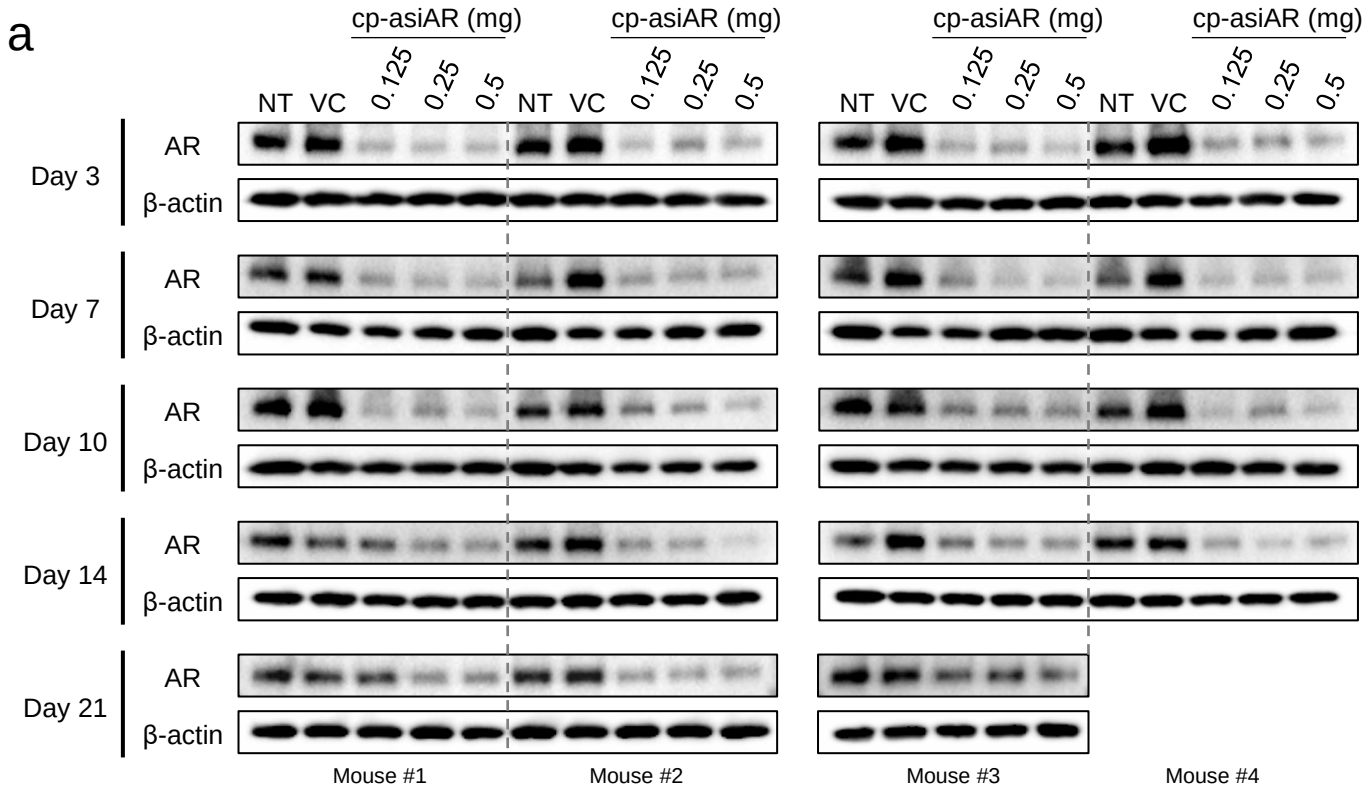


Fig S3. AR protein level by western blot analysis. Injection sites of the dorsal skin were prepared at the indicated time post-injection. Four mice were used at each time point. Mouse #4 belong to at day 21 group become sick for unknown reason (not related to injection) and removed from the analysis.

Fig S4

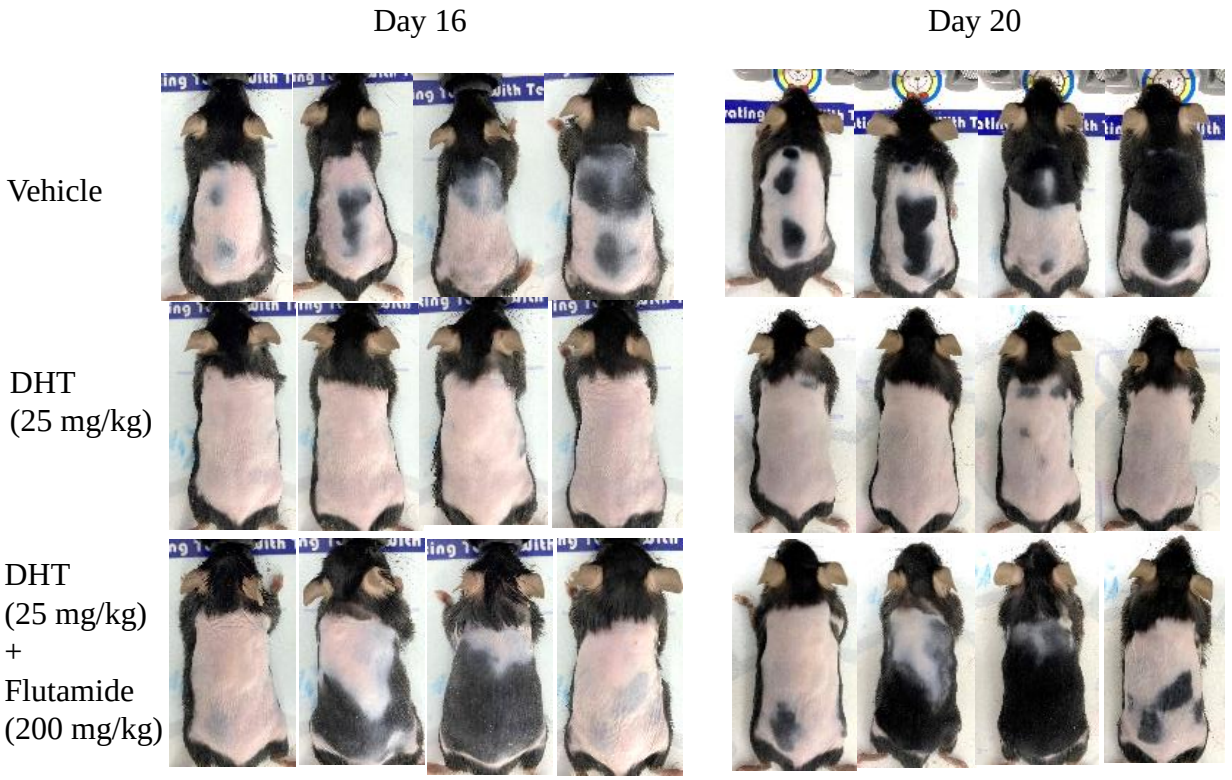


Fig S4. Comparison of hair regrowth on dorsal skin between vehicle and DHT-treated mouse. Vehicle-treated mouse dorsal skin enters active hair regrowth phase during third week after shaving. Hair regrowth is significantly inhibited by subcutaneous injection of DHT. Flutamide co-treated mouse recovered the hair growth efficacy comparable to non-DHT-treated (Vehicle) mouse.

Fig S5

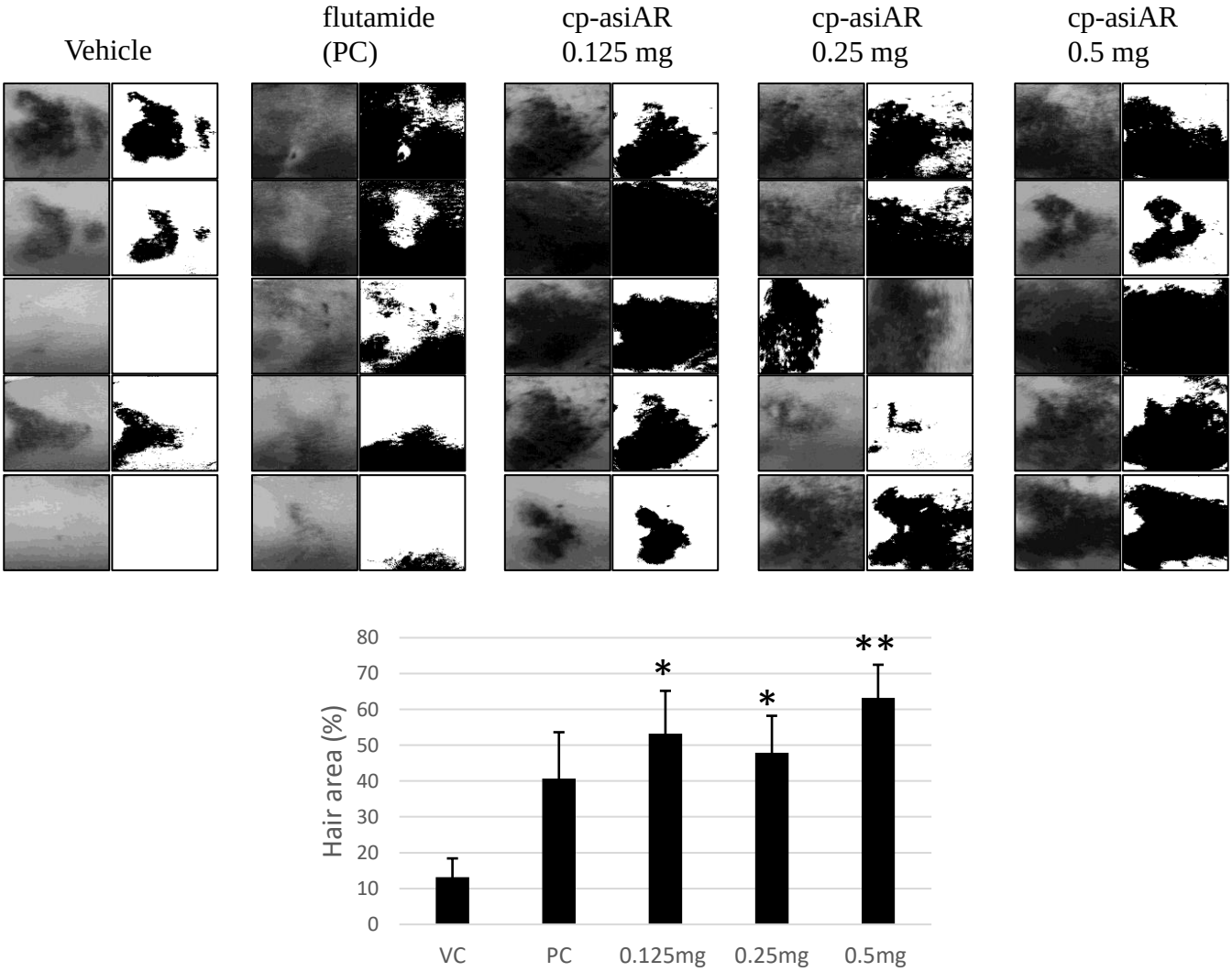


Fig S5. Quantitation of hair regrown area at day 15 by image analysis on dorsal skin between vehicle and DHT-treated mouse.
Dorsal skin images were transformed to gray scale and to black and white image in fixed parameter using ImageJ software. Black areas was quantified in the ImageJ and presented as mean and standard deviation. Statistical significance was calculated using t-test with the vehicle control (*, $p<0.05$; **, $p<0.01$).

Fig S6

G1: Vehicle control
G2: Flutamide 200 mpk
G3: cp-asiAR 0.125 mg
G4: cp-asiAR 0.25 mg
G5: cp-asiAR 0.5 mg

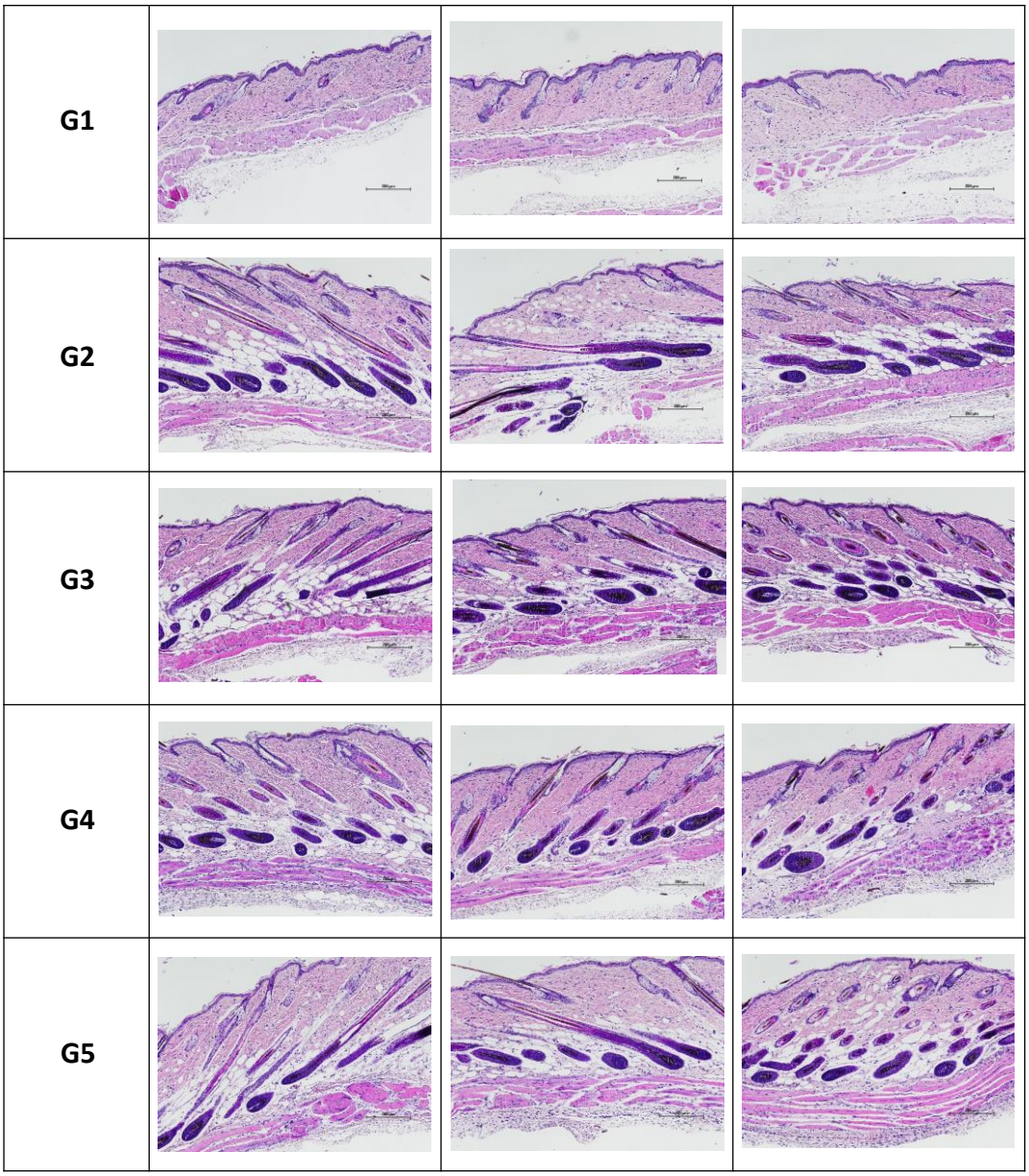


Fig S6. Quantitation of anagen and telogen hair follicles from vertical section of dorsal skin between vehicle, flutamide, or cp-asiAR treated groups.
All groups were treated with DHT. Hair follicles in the subcutaneous layer with bulb shape was counted as anagen. Hair follicles in the dermis layer with dermal papilla was counted as telogen. Total numbers from three images (single image/mouse) were shown in the right. We cannot discriminate the catagen hair from the anagen hair.

Fig S7

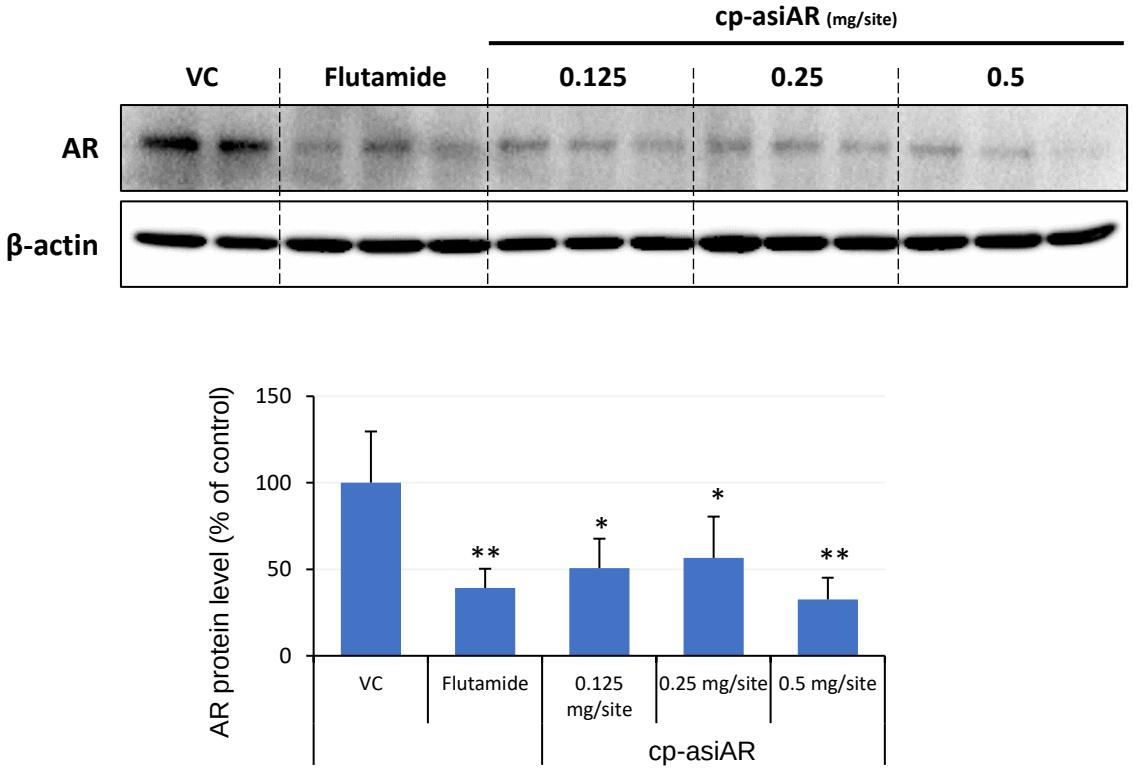


Fig S7. AR knockdown in dorsal skin in AGA models

After hair regrowth experiment (at day 21), dorsal skin was isolated and prepared for AR protein analysis. Three out of fives were shown as representative results. AR protein level relative to the vehicle control was shown as mean and standard deviation (n=5). Statistical significance was calculated by t-test with the vehicle control (*, $p<0.05$; **, $p<0.01$).

Fig S8

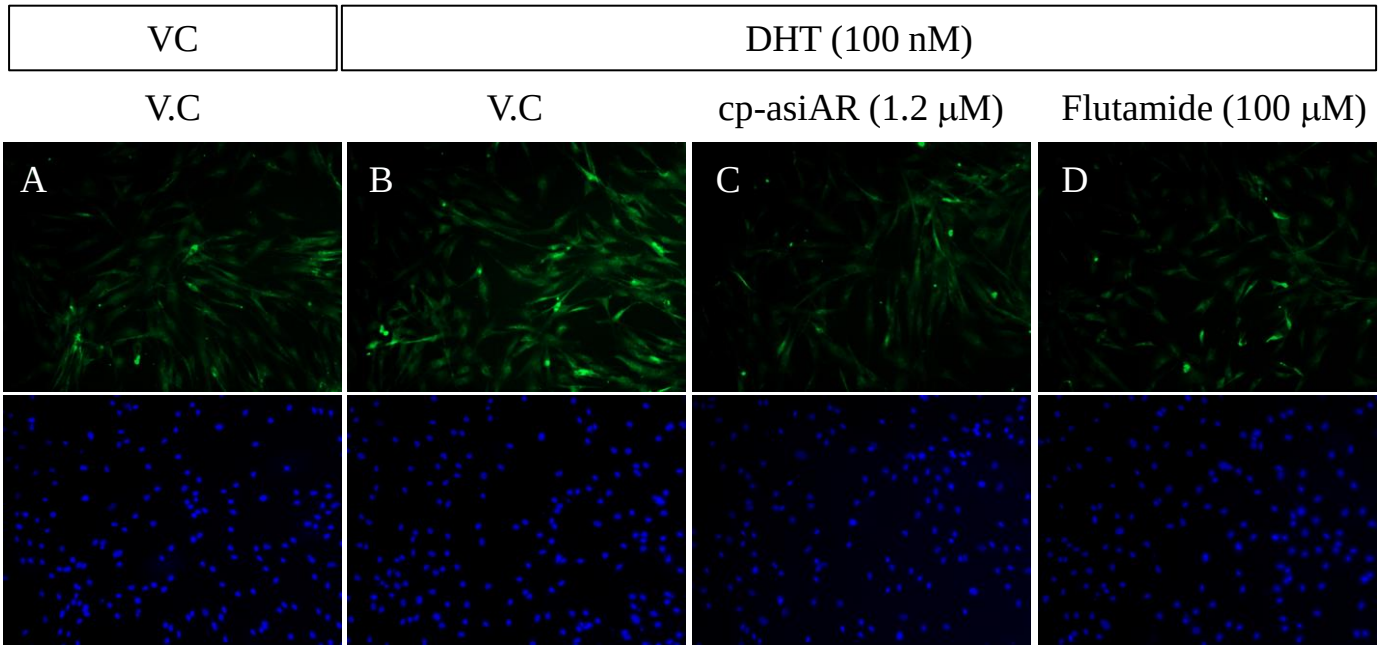


Fig S8. AR reduction by cp-asiAR in human primary DP cells
Human primary DP cells were treated with vehicle alone (A) or treated with vehicle, cp-asiAR or flutamide in the presence of DHT (B-D). At 24 hours after treatment, cells were fixed and immunostained with anti-AR antibodies. DAPI staining image was shown separately (bottom).

Fig S9

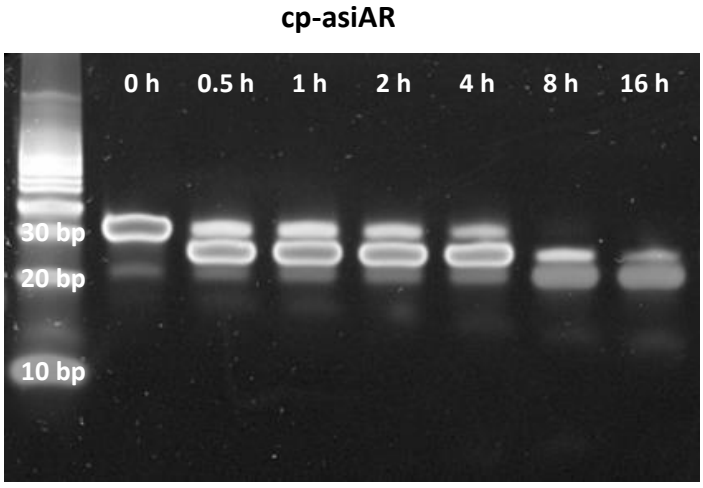


Fig S9. Stability profiling of cp-asiAR
cp-asiRNA duplex were incubated with RNaseA (7.8 μg/ml) for indicated time. After incubation, reaction samples were loaded in the native PAGE with DNA ladder (10 bp).

Table S2

Tissue /time (hr)	0.5	4	8	24	96	168
Skin	1250	516	382	81.4	76.1	34.5
Plasma	2.7	3.1	2.4	0.05	BQL	BQL
Liver	0.9	2.9	4.7	2.7	0.34	0.08
Kidney	0.3	2.8	4.1	4.6	3.5	3.4

Table S2. PK analysis of cp-asiAR in mouse tissues by intradermal injection.
cp-asiAR was injected to three site of dorsal skin intradermally (0.5 mg/site, total 3 sites). Tissues were collected at the indicated time points and cp-asiAR was quantitated by HPLC method. (N=6 for skin, n=2 for other tissues)

Supplemental methods

1. Stability profiling of cp-asiAR

Duplex cp-asiAR (final concentration 1 μM) was incubated with RNase A (final concentration 7.8 μg/ml, 12091021, Invitrogen) at 37˚C and aliquots of mixture was sampled at 0.5, 1, 2, 4, 8, and 16 hours. Samples were electrophoresed in the 15% Native PAGE and stained with Gel-red stain (SCT123, Millipore). RNase A was not included in 0 hour incubation sample.

2. Bioanalysis and pharmacokinetic analysis of cp-asiAR in mouse tissues

The bioanalytical methods used for this study were based on the previous reports[1]. Briefly, peptide nucleic acid (PNA) hybridization assay was coupled to high-performance liquid chromatography (HPLC) with fluorescence detection (FD). The PNA probe complementarily and effectively binds to the antisense strand of the cp-asiAR. The anion exchange chromatography enables the efficient separation of unhybridized PNA and auto-fluorescent materials in tissue from hybridized antisense-PNA. Also, the detection of the fluorescence dye conjugated to the PNA probe improves the sensitivity. These two methods were qualified using rat skin and plasma before the sample analysis. The lower limit of quantification (LLOQ) in the skin is 100 ng/g and LLOQ in plasma is 2.00 ng/mL. The PK parameters were calculated based on the non-compartmental analysis after extravascular administration using PKSolver, an add-in program for pharmacokinetic analysis in Microsoft Excel. The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal method (linear interpolation).

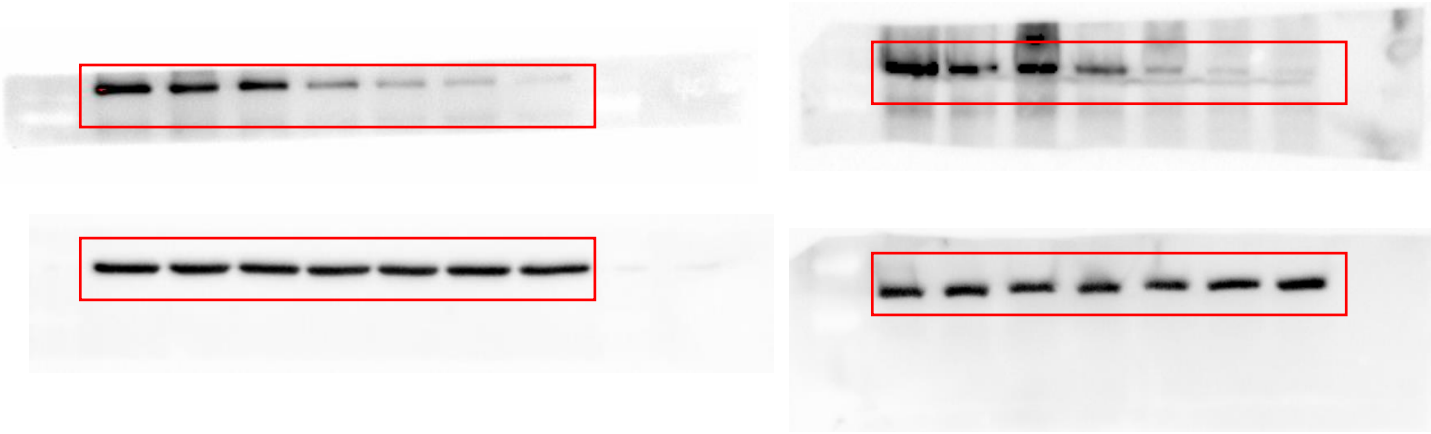
3. Immunostaining of AR in human DP cells

Each group of cells treated with the corresponding agent(s) were washed with PBS and fixed using 10% formalin solution (HT501128; Sigma-Aldrich, Saint Louis, MO). They were sequentially reacted with Permeabilization buffer. 1x (00-8333-56; Invitrogen, Waltham, MA, USA) and AR antibody (Sc7305; Santa Cruz, Dallas, TX, 1:50), and then with Alexa Fluor™ 488 mouse (A21202; Thermo, Waltham, MA, USA, 1:500). Staining of the nucleus was done using DAPI (62248; Thermo, Waltham, MA, USA). Fluorescence Mounting Medium (s3023; DAKO, Glostrup, Hovedstaden, Denmark) was added before mounting the cover glass. Images were obtained using Fluorescence microscope (Carl Zeiss, Land Baden-Württemberg, Oberkochen, Germany).

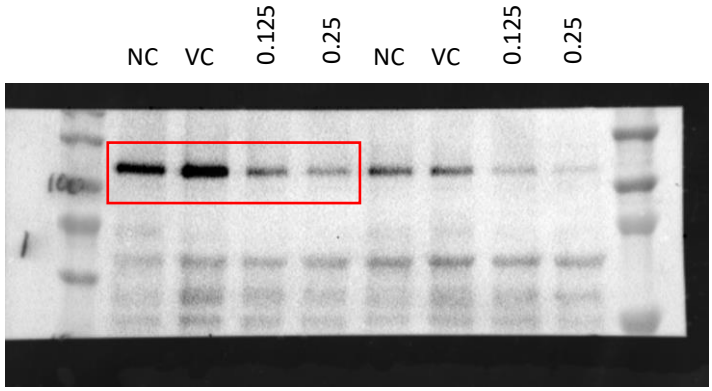
(1) Tian, Q.; Rogness, J.; Meng, M.; Zheng, L. Quantitative determination of a siRNA (AD00370) in rat plasma using peptide nucleic acid probe and HPLC with fluorescence detection. *Bioanalysis* **2017**, 9 (11), 861-872. DOI: 10.4155/bio-2017-0017

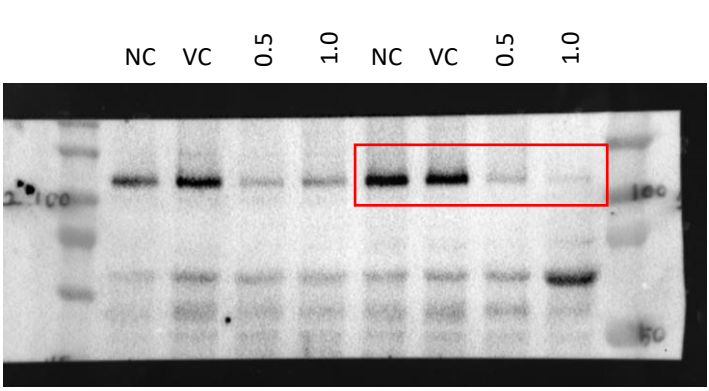
Uncropped WB images

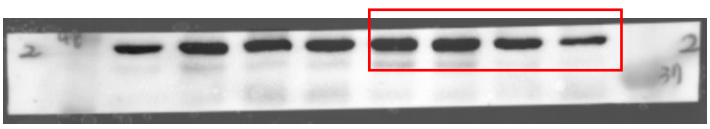
Complete WB images for Figure 1.
(Top) AR western blot images were shown. Red box was cropped.
(Bottom) b-actin western blot images were shown. Red box was cropped.



Complete WB images for Figure 2B.
(Top) AR western blot images were shown. Red box was cropped.
(Bottom) b-actin western blot images were shown. Red box was cropped.



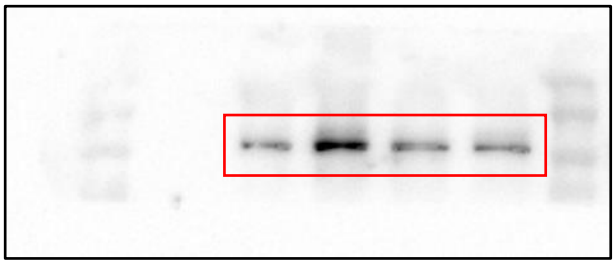




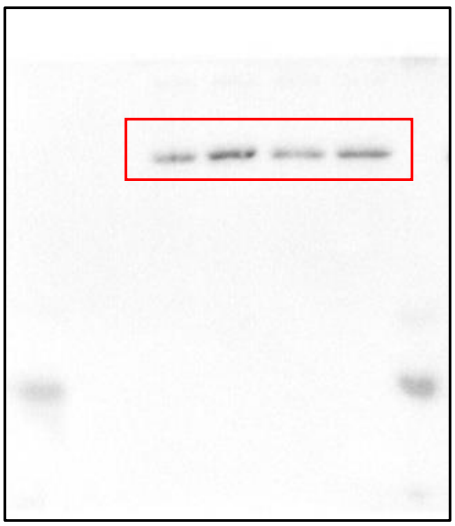
Uncropped WB images

Complete WB images for Figure 5B.
AR, DKK-1, IL-6, TGF-β1 and β-actin western blot images were shown. Red box was cropped.

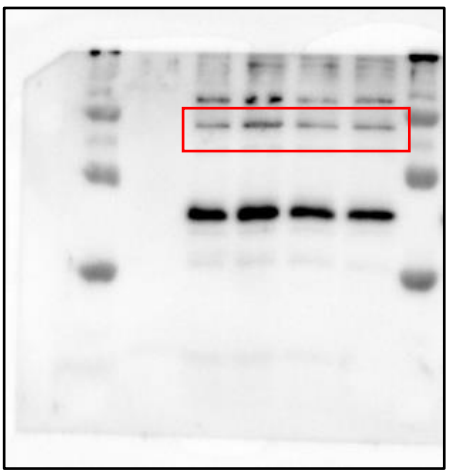
AR



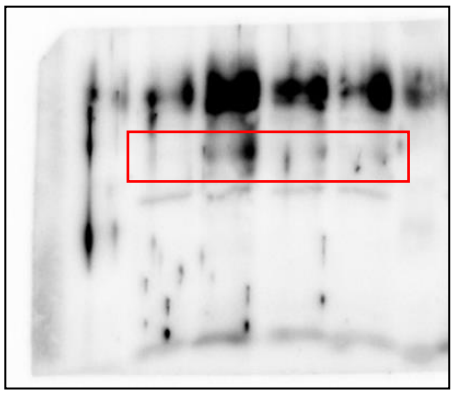
DKK-1



TGF-β1



IL-6



β-actin