Supporting Information Online

The Ascorbate Synthesis Pathway: Dual Role of Ascorbate in Bone Homeostasis

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Mouse Chow Diet Preparation: We determined that, on average, mice eat approximately 2.5 grams of mouse chow per day. In the case of vitamin C, we prepared dried mouse chow pellets containing 1% vitamin C. The pellets were prepared by adding 100ml containing 10 gms Vitamin C (Aldrich A9290-2) dissolved in 100 ml water to a solution of 10 gms Bacto agar (DIFCO Lab) dissolved in 800 ml hot water. The solution was mixed with 1000 gms of powdered mouse chow in a blender to make a thick paste that was flattened into a sheet ½ inch thick and cut into 2" squares and dried in a dehydrator oven at 85º F. until it returns to its original weight. These chow pellets assay at ~0.65% vitamin C due to loss of vitamin C in the preparation process. A compressed 1% Vitamin C chow pellet diet prepared for us by Harlan (Teklad TD.07727) also assayed at ~0.65% Vitamin C. On average these diets deliver a dose of ~25 mg vit C/mouse/day (1g/kg body wgt/day). Vitamin C was undetectable in regular mouse chow pellets provided by Harlan. The pycnogenol chow pellets were similarly prepared with 500 mg Pycnogenol (a generous gift from Horphag Research UK Ltd.) to give a concentration of 0.05% resulting in a dose of ~1.25 mg pycnogenol/mouse/day (50 mg/kg body wgt/day). Resveratrol (200 mg in 1000 gms chow = 0.02%) resulted in a daily dose of ~0.5 mg/mouse/day (20 mg/kg body wgt/day). All mouse experiments were approved by the Baylor Institutional Animal Care and Use Committee.

Figure Legends

Figure S1: Schemata for generation of mouse aldehyde reductase knockout

Figure S2: Schemata for the generation of a knock-in mouse carrying an enhanced aldose reductase enzyme (V280L and C298V), and a further derivative mouse aldose reductase knockout.

Figure S3. CAT scans (100 um) showing the "rachitic rosary" (arrows) in a ♂ GULOKO and an AR/GRKO$^-$ double knockout (60 days of age). The ball-like swellings at the costochondral junctions of the ribs (arrows) are only seen in these animals and result from hemorrhage and callus formation due to profound ASC deficiency. The knees and distal tibia are also "swollen". The WT GULOKO littermate is larger and the rib ends are smooth.

Figure S4: Section of nasal turbinate from a WT mouse 3 weeks after castration and fed a regular mouse chow diet. The dystrophic cell masses seen in comparably treated castrated GRKO mice (MS Fig. 3) are never seen in the castrated WT mice.

Figure S5: Kinetics of glucuronate reduction by recombinant murine aldose (AR) and aldehyde reductase (GR) enzymes. The Km's for glucuronate are similar in both enzymes, with the major difference being the superior catalytic efficiency, *k*cat, of GR (~100-150 fold) due to the unique presence of 2 arginine residues (R308 and R311) in the C-terminal loop that bind the carboxyl group of glucuronate (1). Note that a 1:1 molar mixture of AR and GR predominantly reflects GR enzymatic activity.

Reference List

1. Barski, O. A., Gabbay, K. H., and Bohren, K. M. (1996) *Biochemistry* **35,** 14276-14280

Figure S1

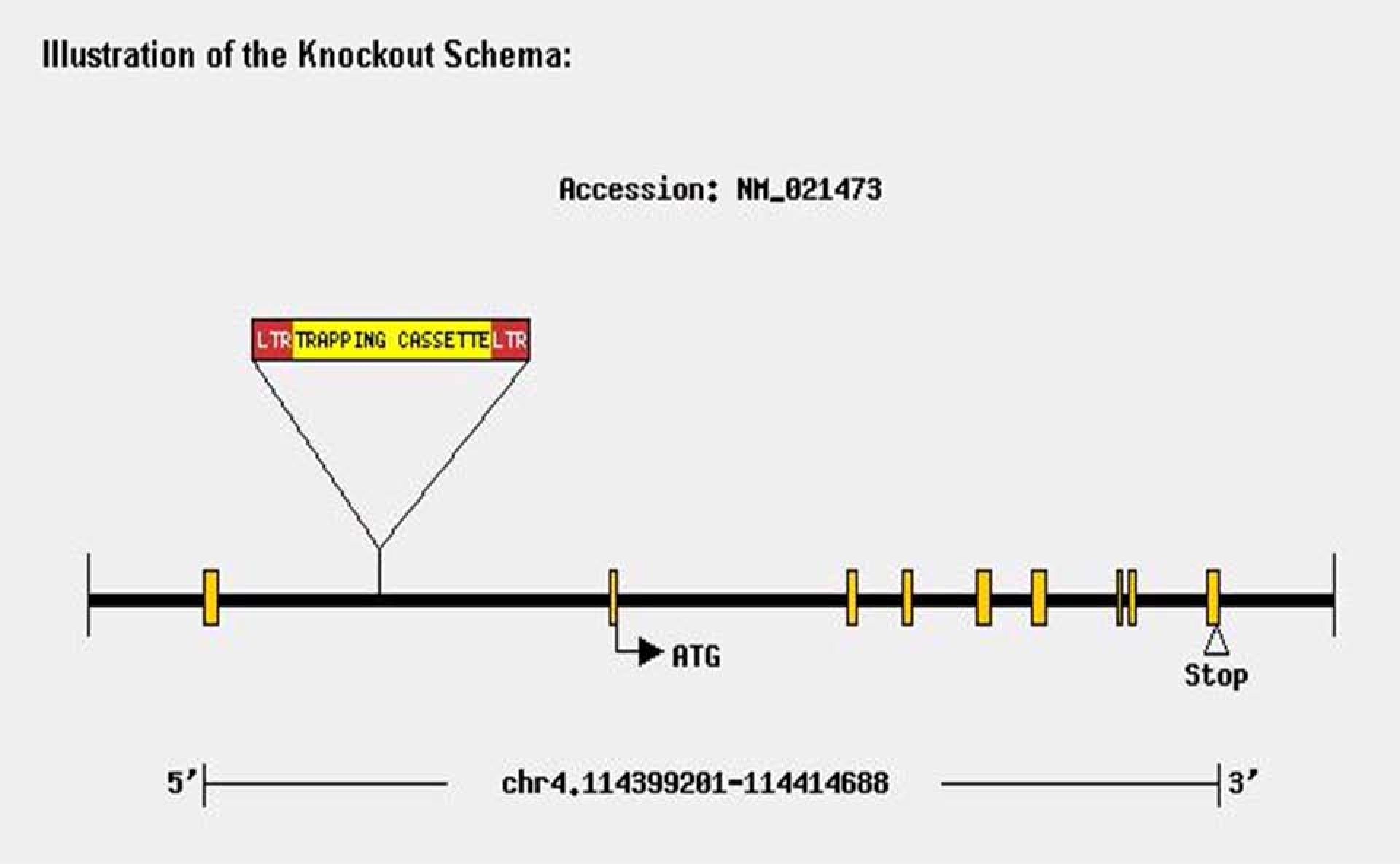
Illustration of the Knockout Schema:
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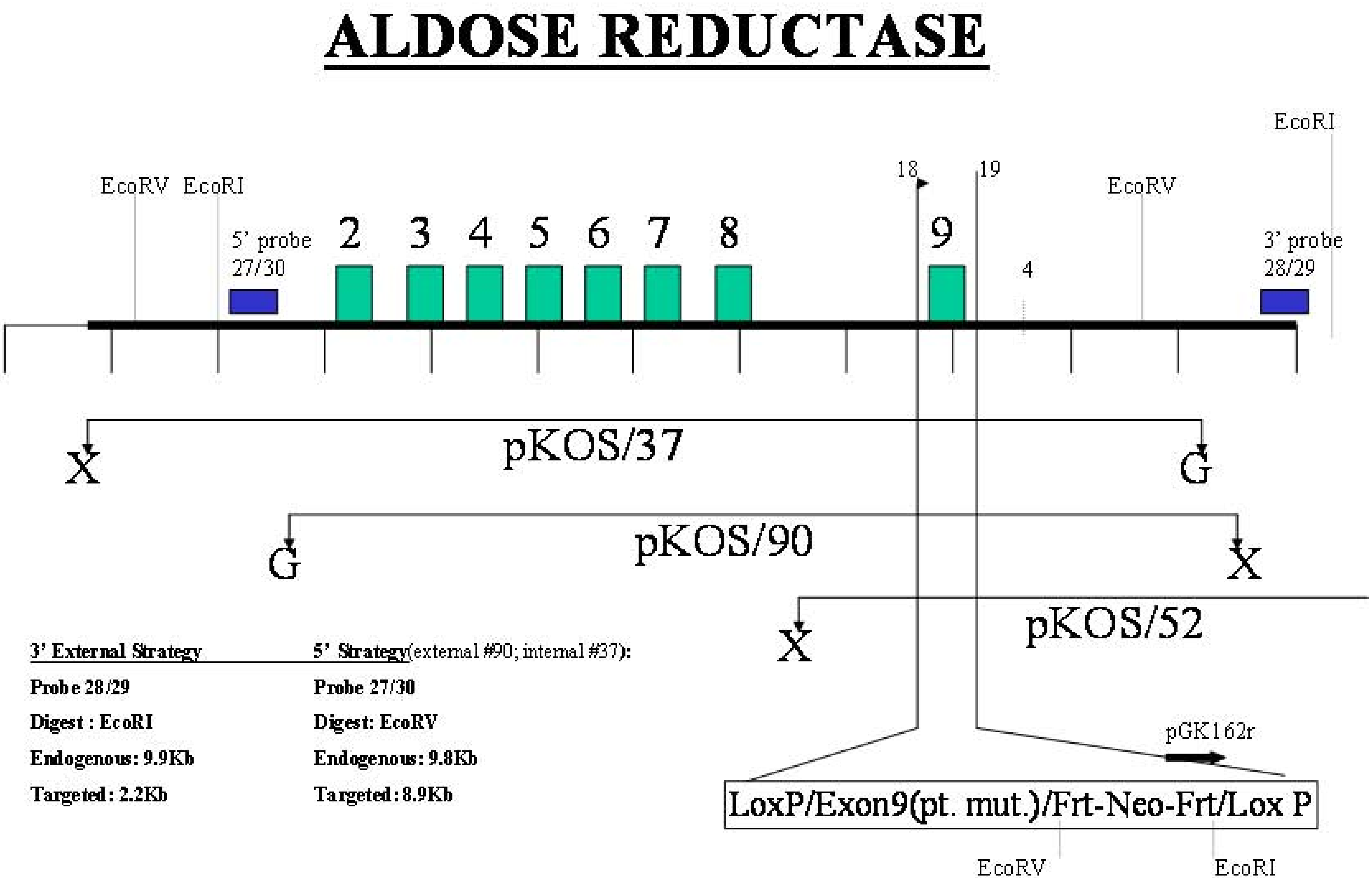
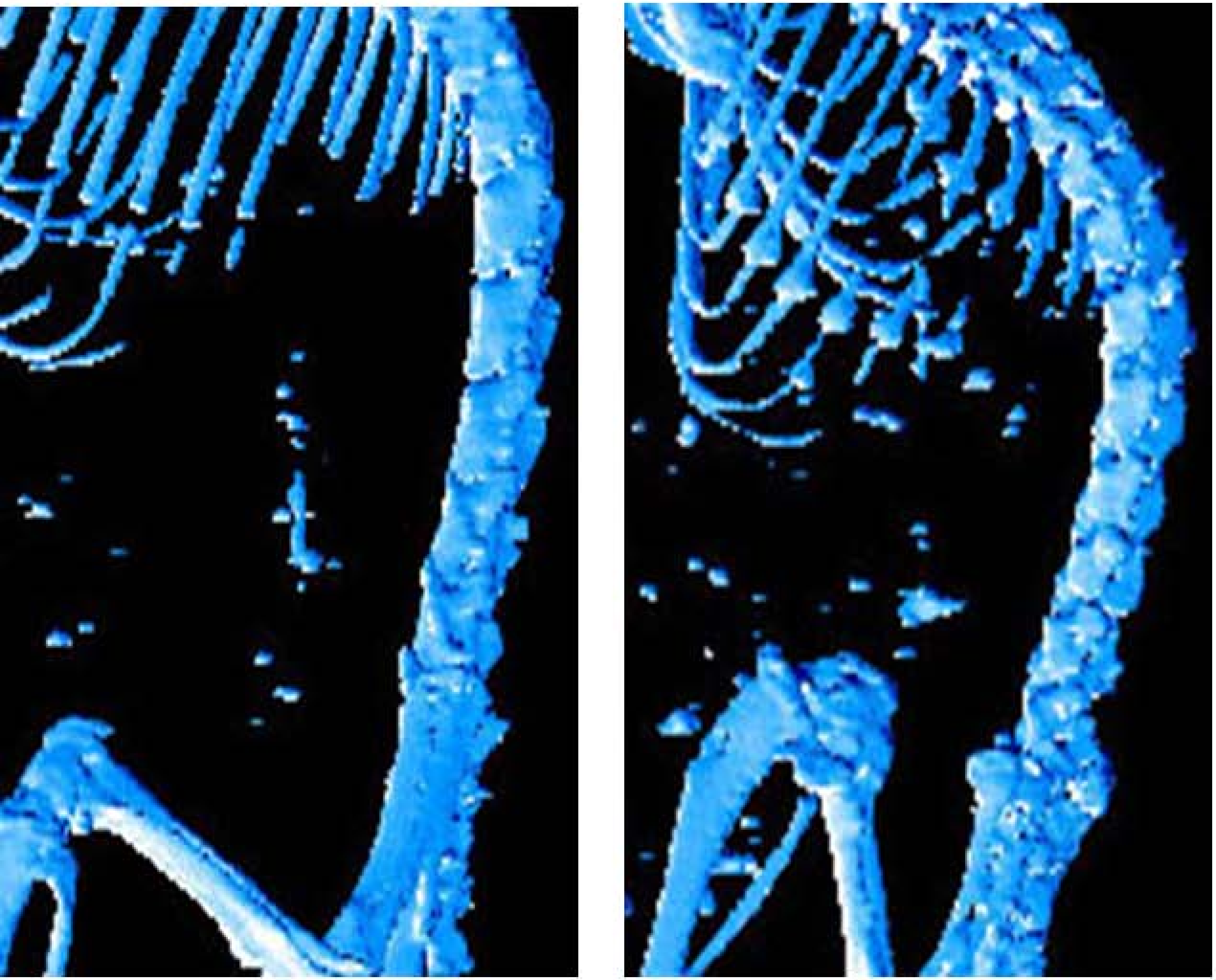
LTR TRAPPING CASSETTE LTR
ATG
Stop
5'
chr4.114399201-114414688
3'

Figure S2



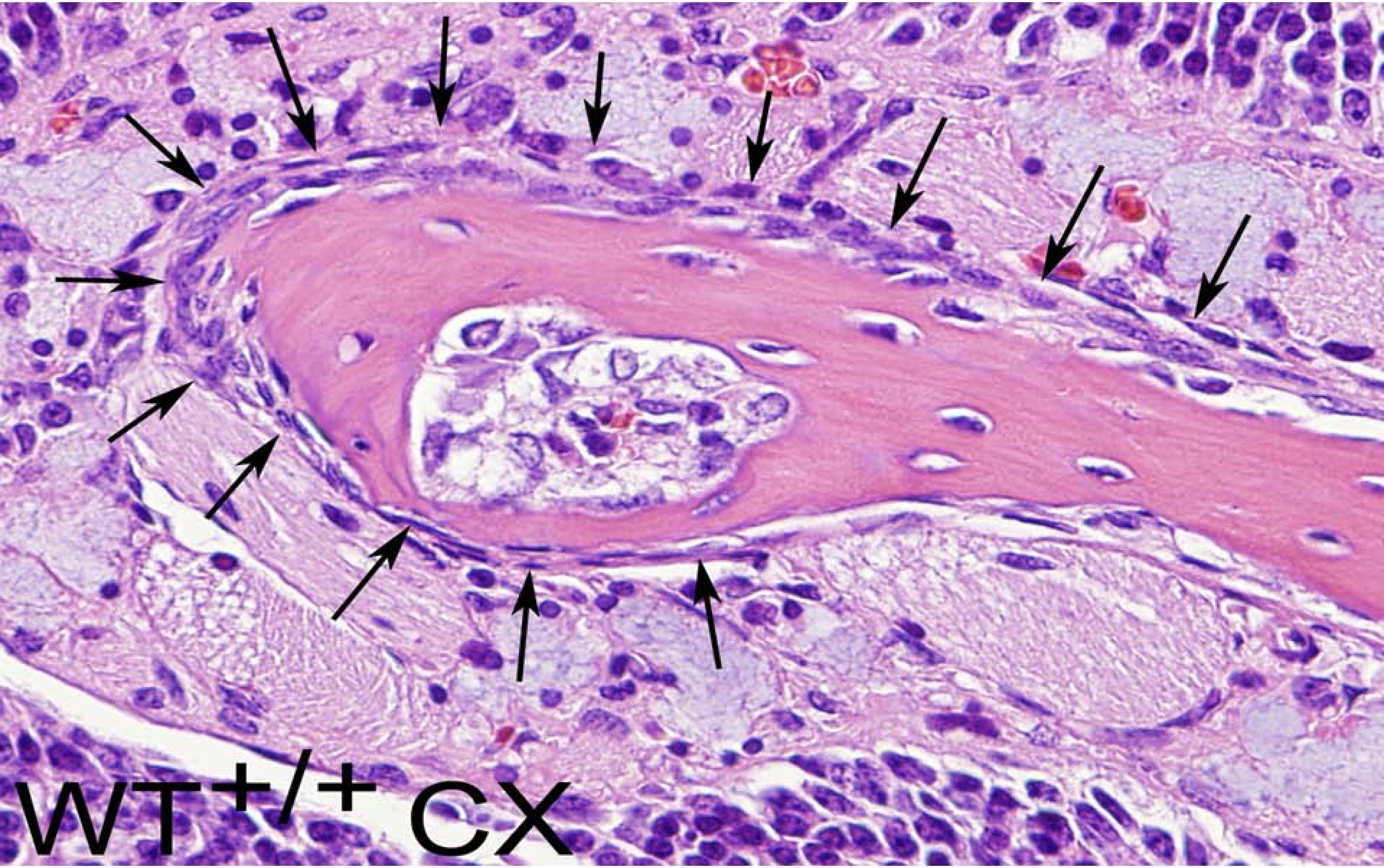
ALDOSE REDUCTASE
EcoRV
EcoRI
EcoRV
EcoRI
18
19
EcoRV
5' probe
27/30
2
3
4
5
6
7
8
9
4
3' probe
28/29
pKOS/37
X
G
pKOS/90
G
X
pKOS/52
X
3' External Strategy
Probe 28/29
Digest : EcoRI
Endogenous: 9.9Kb
Targeted: 2.2Kb
5' Strategy(external #90; internal #37):
Probe 27/30
Digest: EcoRV
Endogenous: 9.8Kb
Targeted: 8.9Kb
pGK162r
LoxP/Exon9(pt. mut.)/Frt-Neo-Frt/Lox P
EcoRV
EcoRI

Figure S3



WT **GULOKO GULO$^{-/-}$** **AR/GRKO AR$^{-/-}$/GR$^{-/-}$**

Figure S4



Glucuronate Reduction by Aldehyde & Aldose Reductase

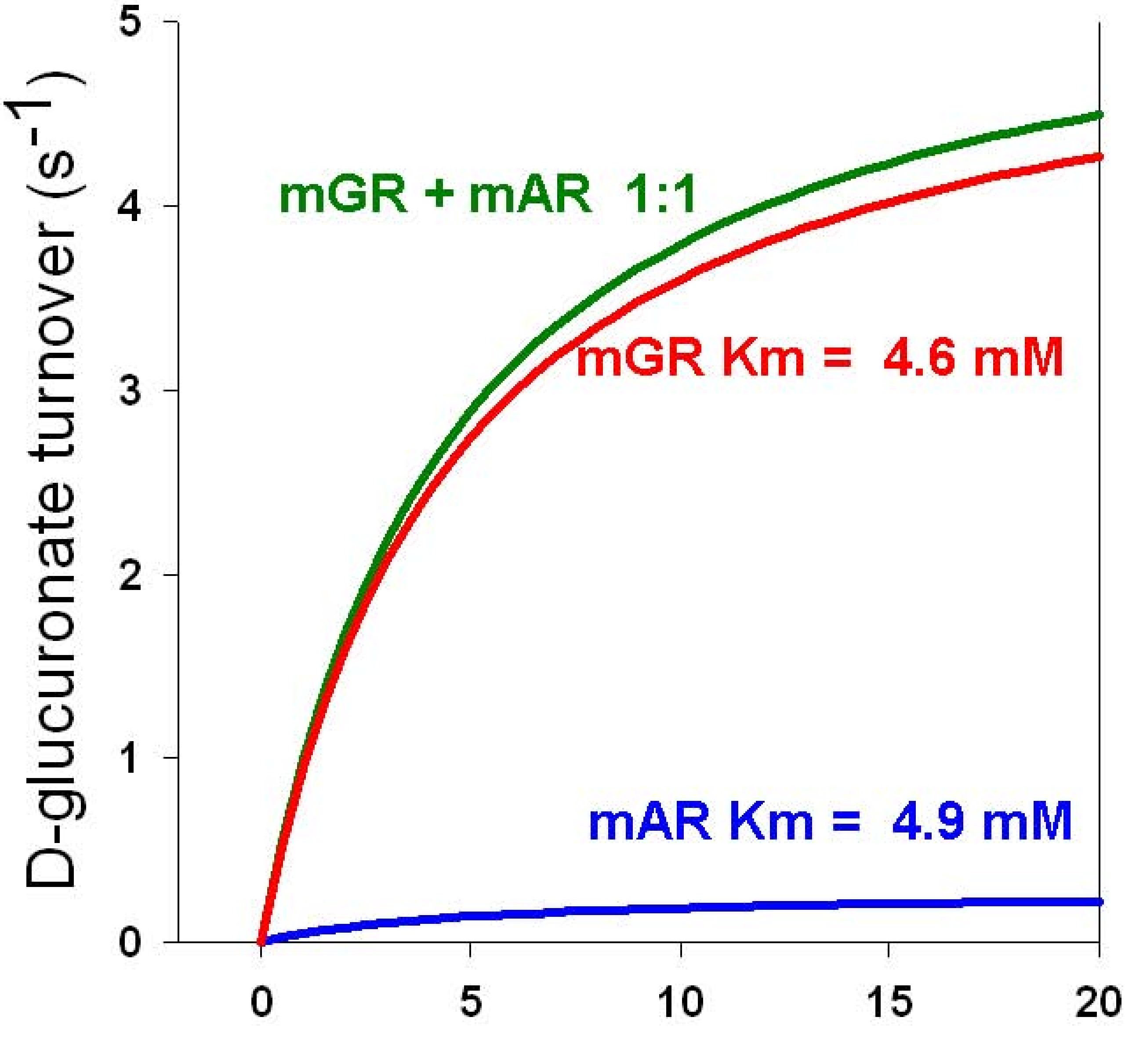


Figure S5