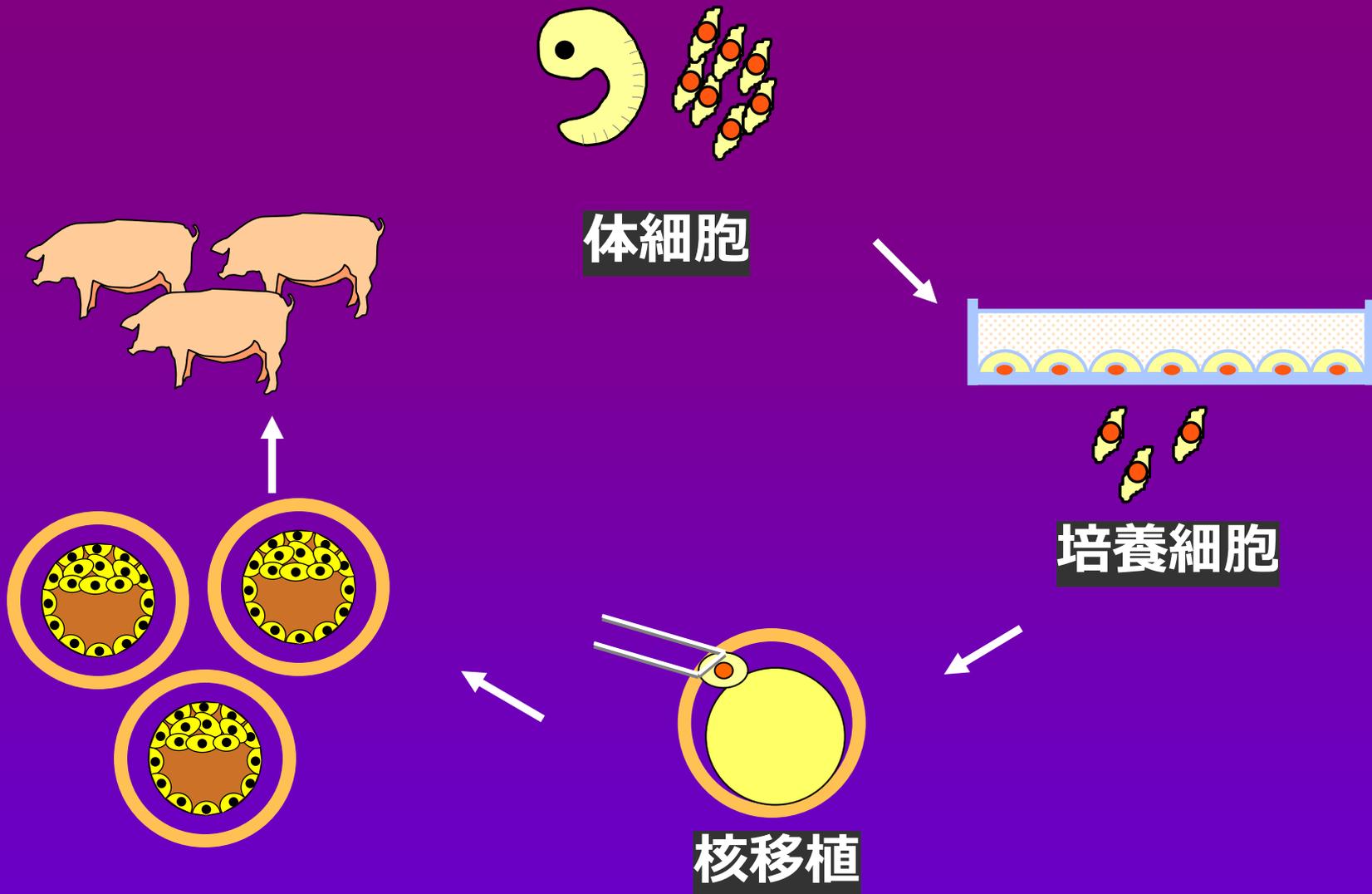
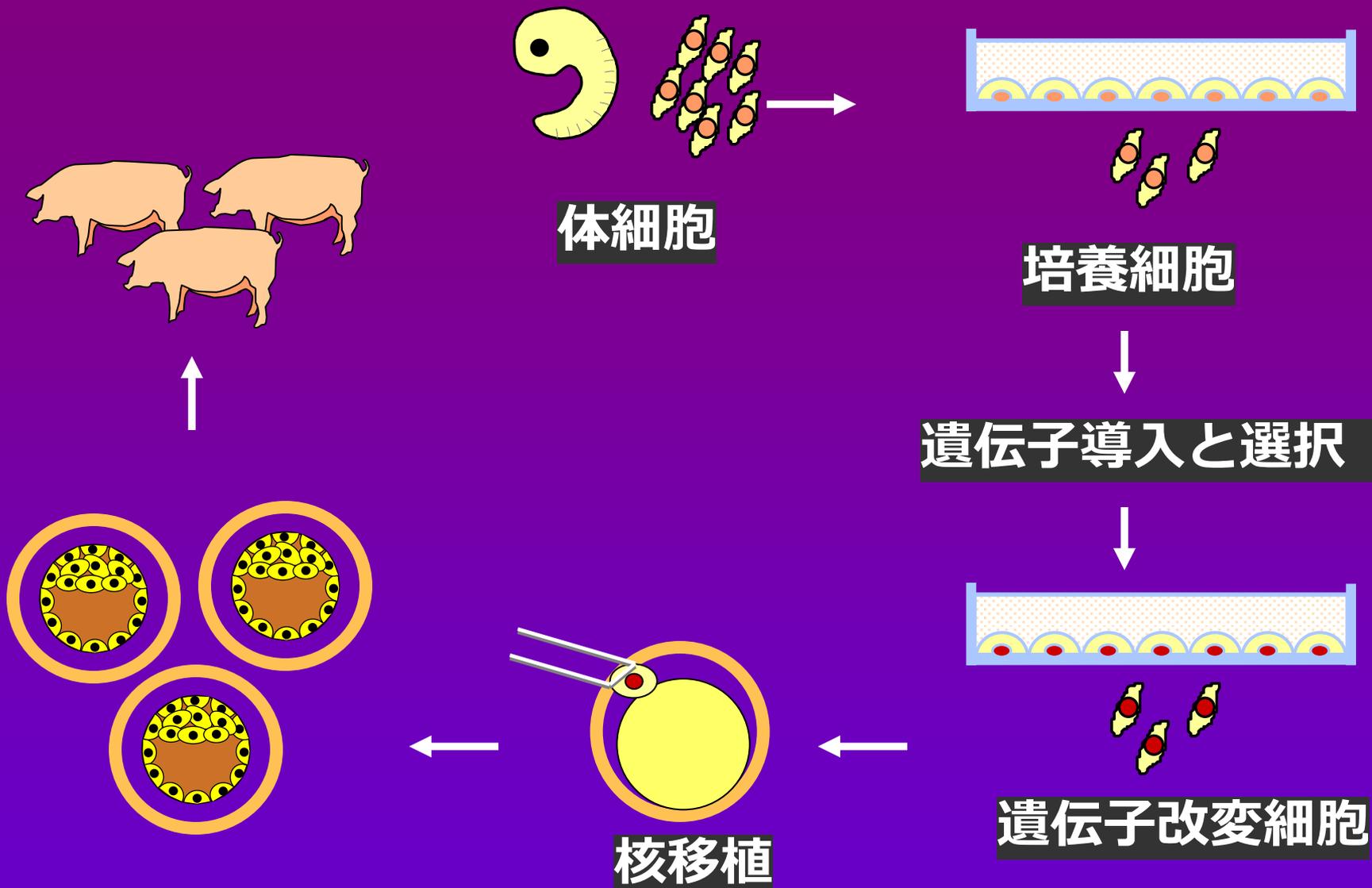


# 体細胞核移植技術を用いた 遺伝子改変ブタの作出



体細胞核移植によるクローンブタの生産



体細胞核移植による遺伝子改変クローンブタの生産

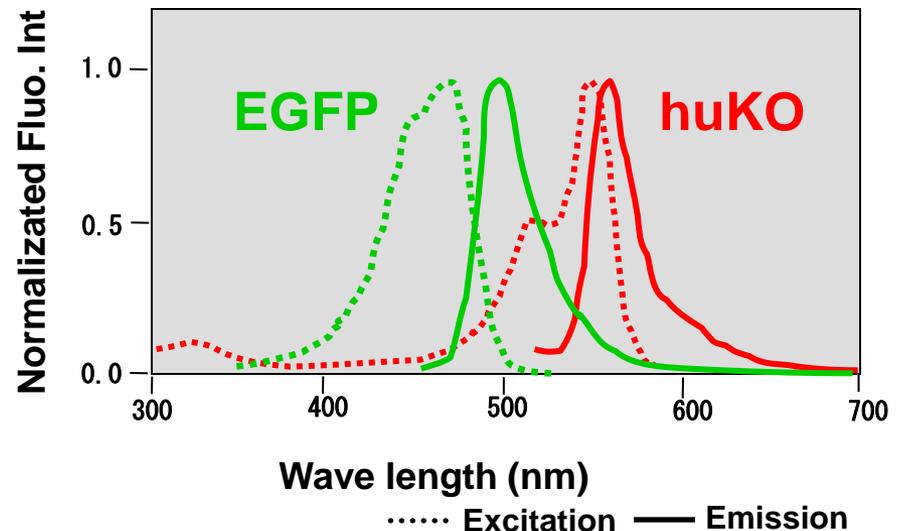
# 赤色蛍光タンパク遺伝子導入ブタの作出

## **Kusabira Orange** (Karasawa *et al.*, Biochem.J.,2004)

- ◆ ヒラタクサビライシ (*Fungia concinna*) 由来の赤色蛍光タンパク
- ◆ humanized Kusabira – Orange (huKO) は Codon Usage をヒト型に変更したもの
- ◆ huKO は蛍光特性が DsRed に近い赤色蛍光タンパク
- ◆ huKO は蛍光波長が長い → バックグラウンドの影響受けにくい

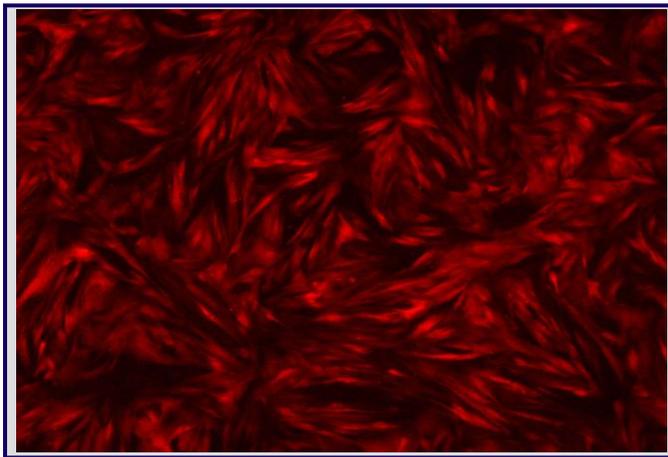
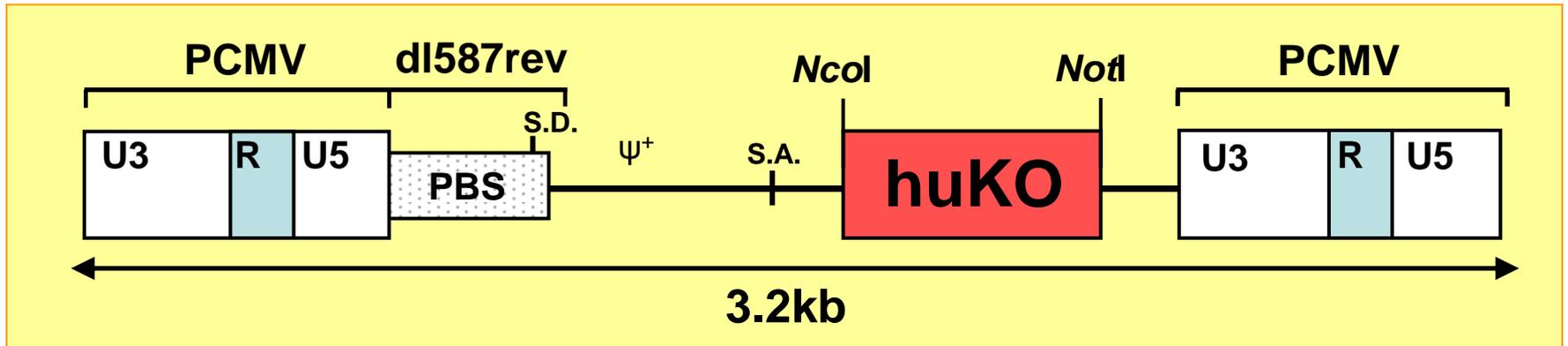
### Excitation/ Emission

huKO	548 / 559 nm
EGFP	488 / 507 nm
DsRed	558 / 583 nm

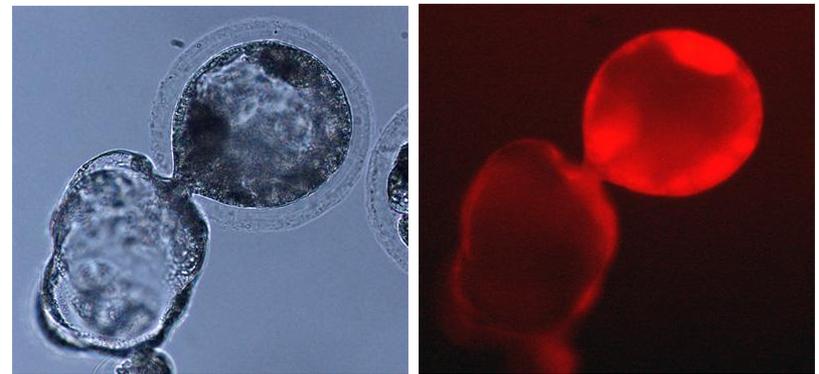


# ドナー細胞へのhuKO遺伝子導入

## ◆ レトロウイルスベクター: DΔNsaphuKO



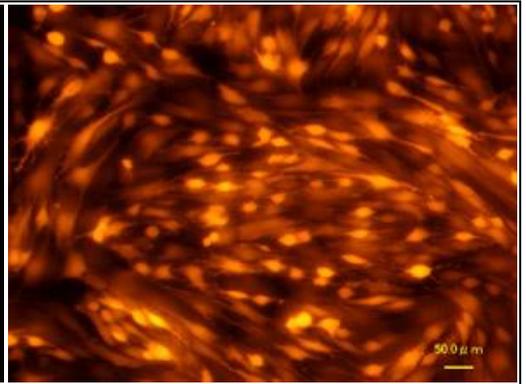
遺伝子導入後の胎仔繊維芽細胞



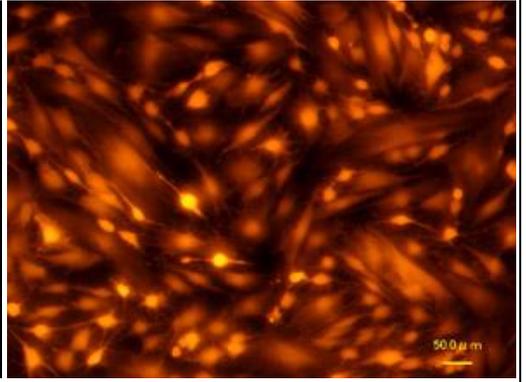
クローン胚盤胞におけるhuKO発現

# クローン胎仔におけるhuKO発現(妊娠47日目)

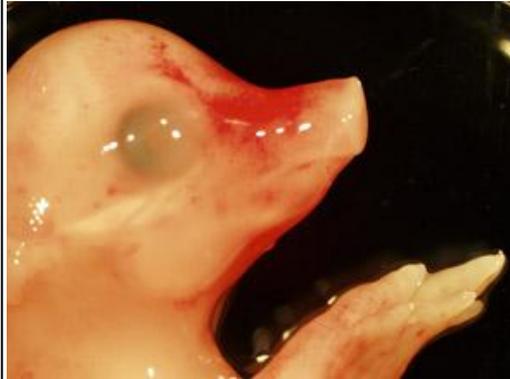
No. 1



No. 2



No. 3



明視野像

蛍光観察像

細胞での蛍光観察像

# クサビラオレンジ遺伝子導入トランスジェニックブタの作出

レシピエントNo.	移植胚数	妊娠/分娩	胎仔/産仔数 (死産仔数)
1	76	+(開腹)	3
2	119	+	3(0)
3	94	+	6(2)
4	93	+	5(0)
5	123	+	4(2)
合計	505	100%	21(4) [4.2%]

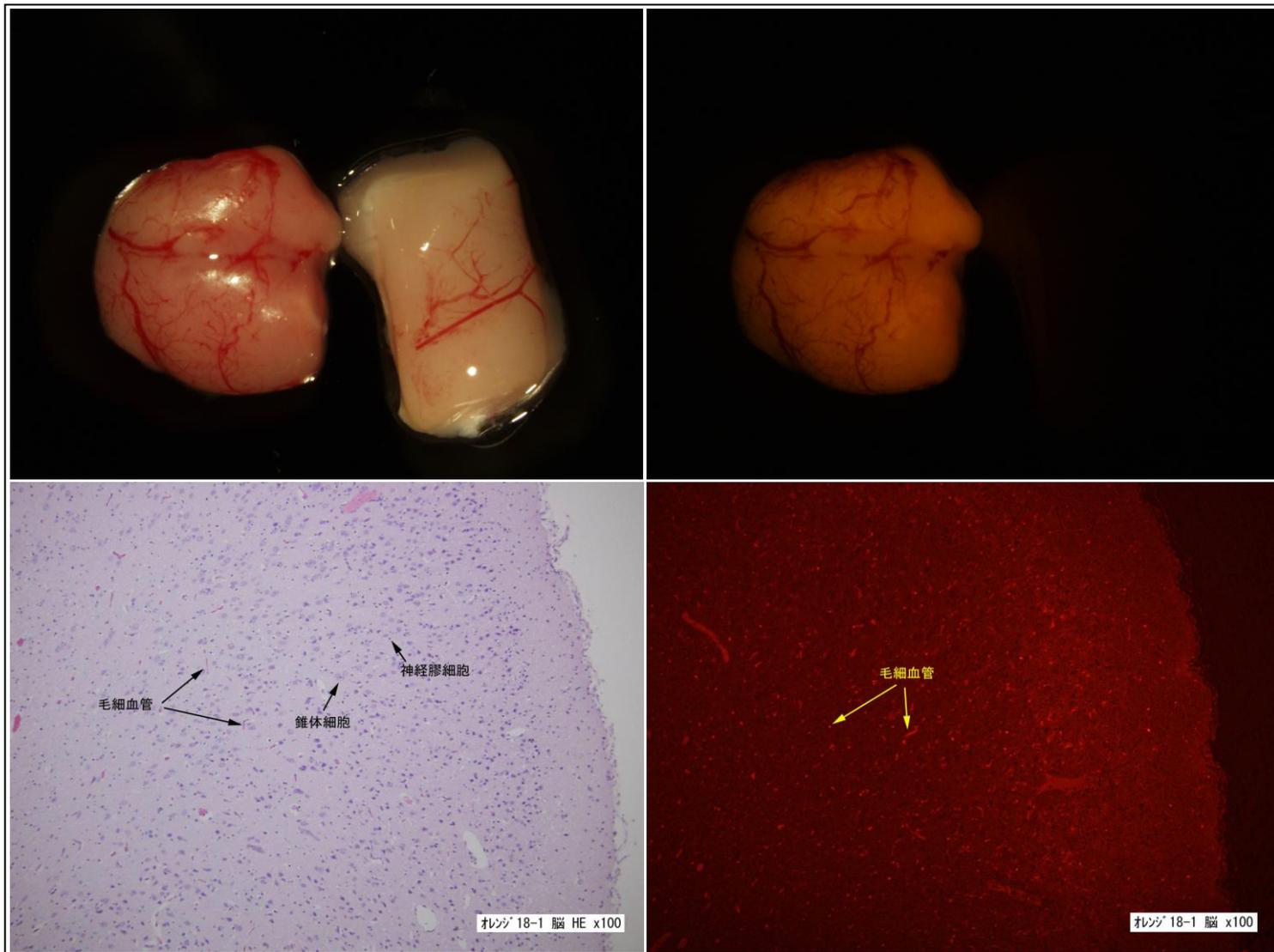
# クサビラオレンジクローン産仔



## 《クローン産仔の解析》

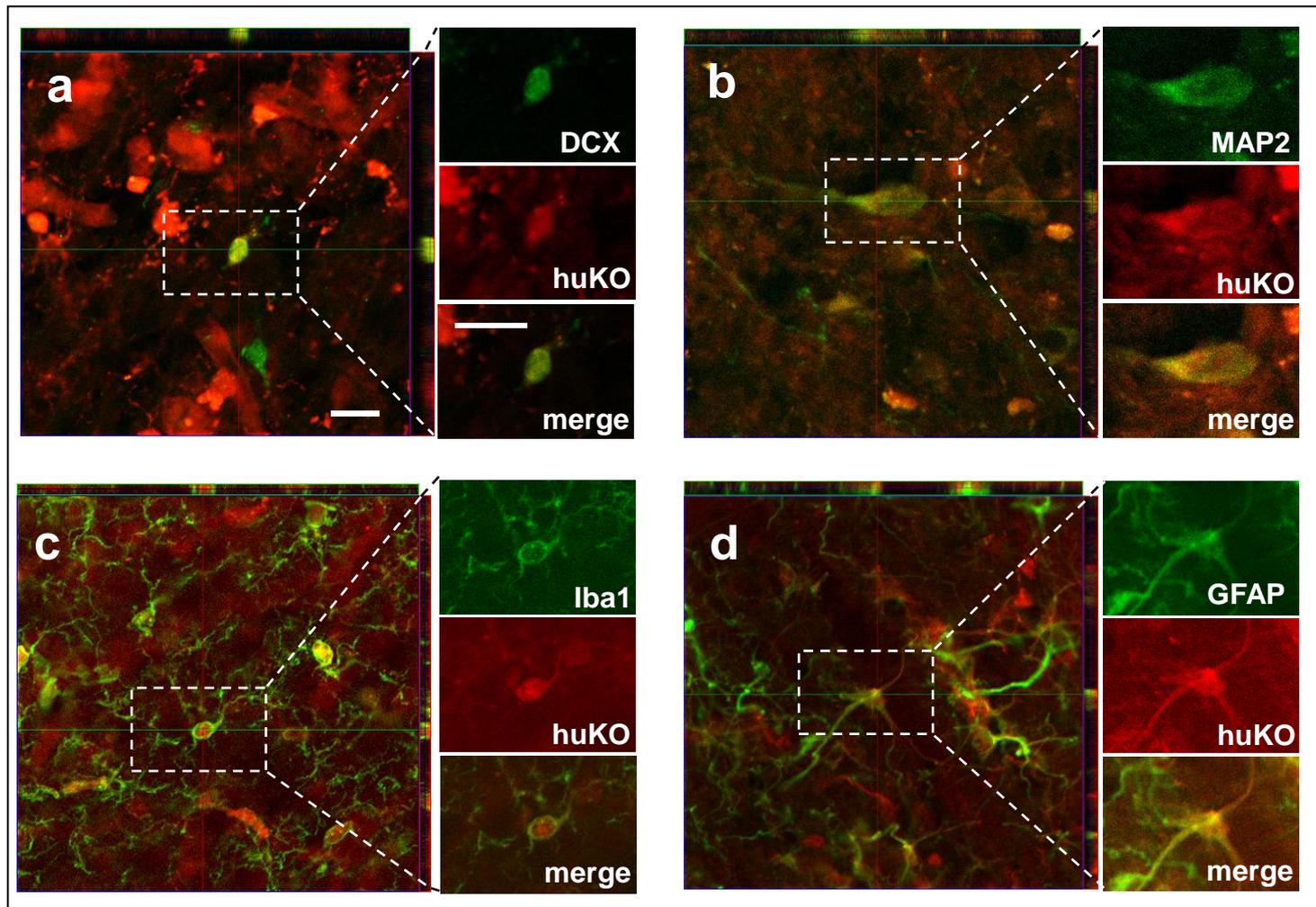
1. サザンブロットィングによる組込み遺伝子コピー数の解析
2. 全身の23臓器・組織—脳・心臓・肺・胃・腸・肝臓・膵臓・脾臓・腎臓・膀胱・生殖器(卵巣・子宮)・皮膚・皮下脂肪・骨格筋・骨・軟骨・滑膜・唾液腺・口腔粘膜・舌・眼球・蹄について、蛍光実体顕微鏡による組織片の蛍光観察
3. 上記の臓器・組織の組織切片を作製し、蛍光観察

# huKOブタ臓器・組織の蛍光発現:脳



明視野像

蛍光発現像

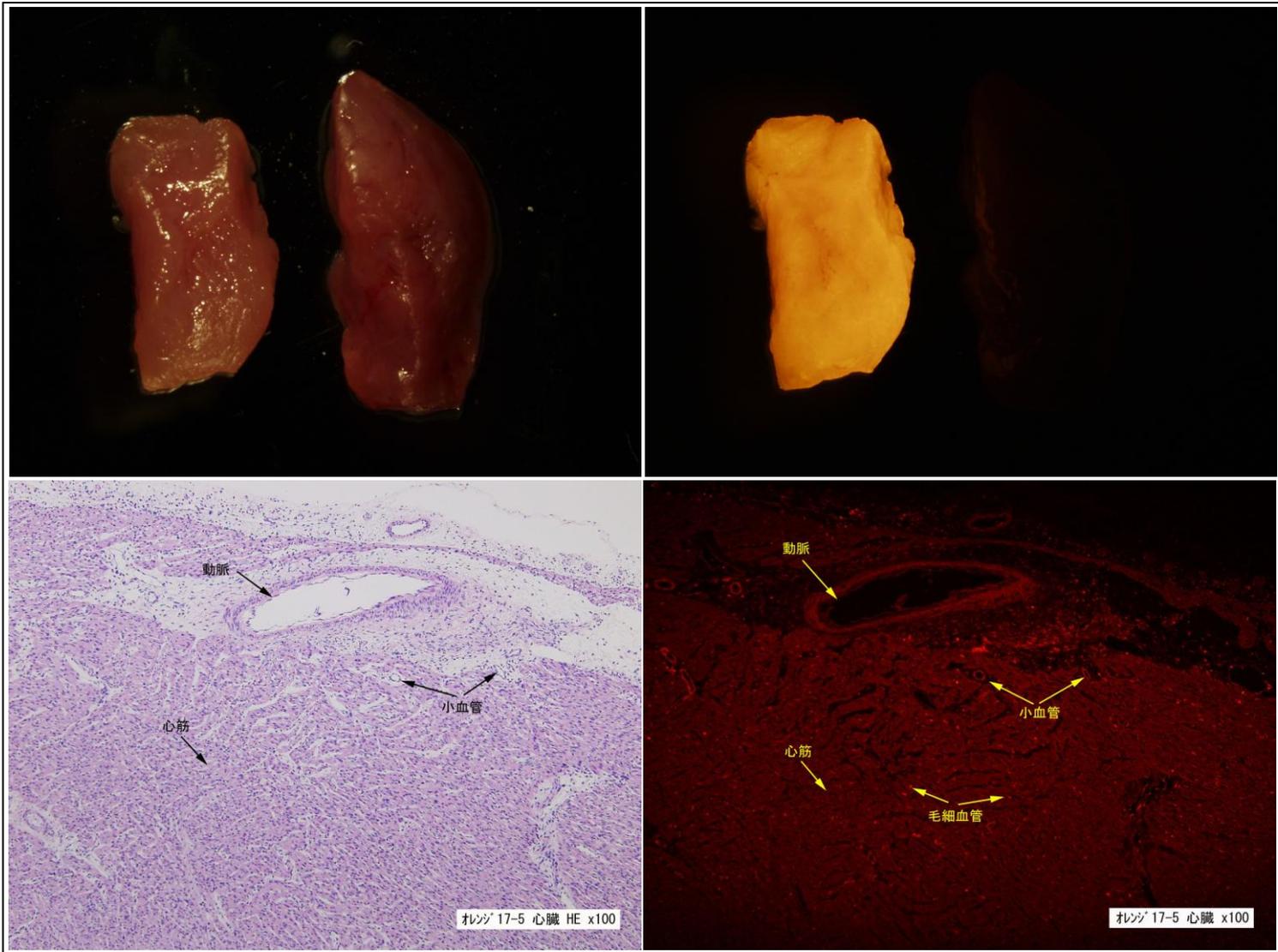


**Supplementary Figure 3.** Immunofluorescent staining of neural cells from huKO transgenic cloned pigs. Cryosections of brain tissue were subjected to immunofluorescent staining using anti-doublecortin (DCX) antibodies (polyclonal goat IgG, Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-microtubule-associated protein (MAP2) antibodies (monoclonal mouse IgG, Upstate Co., Charlottesville, VA), anti-ionized calcium binding adaptor protein (Iba 1) antibodies (polyclonal rabbit IgG, Wako, Osaka, Japan) and anti-glial fibrillary acidic protein (GFAP) antibodies (polyclonal rabbit IgG, generous gift from Dr. Haruhiko Akiyama, Psychiatric Research Institute of Tokyo, Tokyo, Japan) with FITC-labeled secondary antibodies. Laser-scanning microscopy (LSM-510, Carl Zeiss, Germany) showed that huKO expression was colocalized with DCX (a), MAP2 (b), Iba1 (c), and GFAP (d), which are markers for **neuron progenitors**, **mature neurons**, **microglia** and **astrocytes**, respectively (boxed inserts). Preparation of cryosections and immunofluorescent staining was carried out as described elsewhere<sup>1,2</sup>. Scale bar=20  $\mu$ m.

1. Yamada, M. et al. *Neuroscience* **124**, 173-181 (2004).
2. Hayakawa, H. et al. *Neurosci. Res.* **57**, 393-398 (2007).

(順天堂大学 望月秀樹先生より)

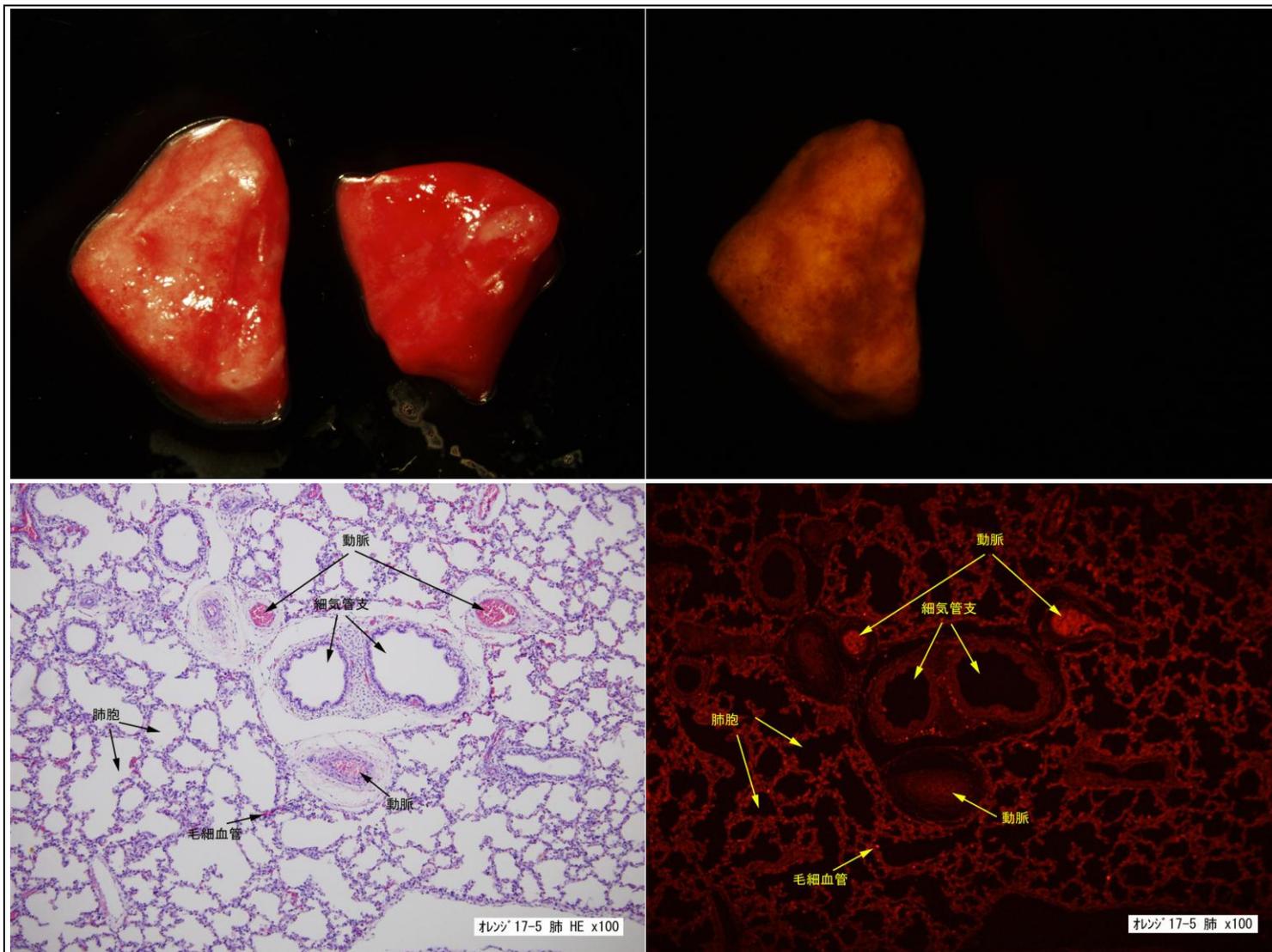
# huKOブタ臓器・組織の蛍光発現: 心臓



明視野像

蛍光発現像

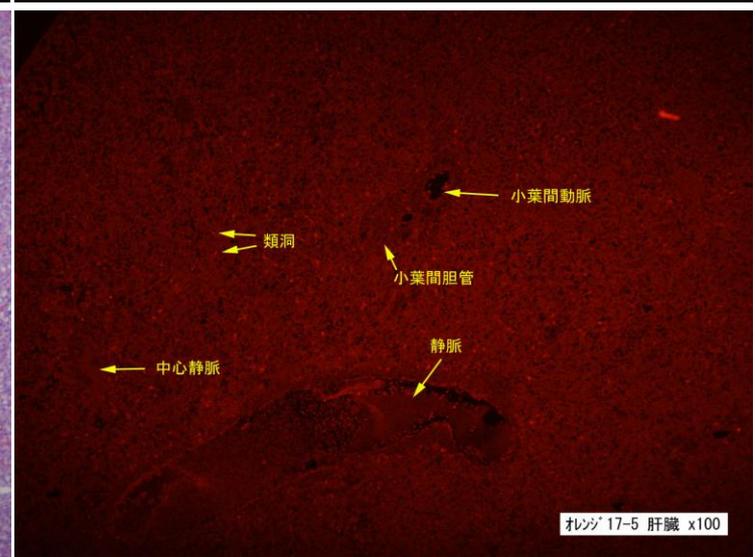
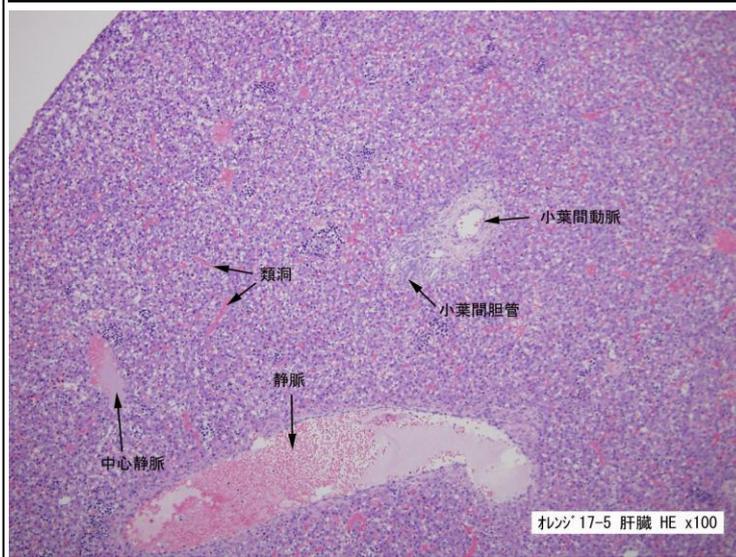
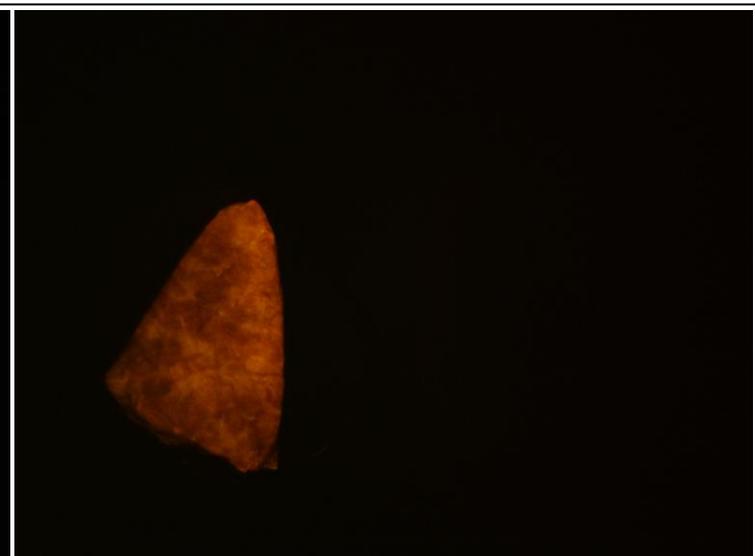
# huKOブタ臓器・組織の蛍光発現:肺



明視野像

蛍光発現像

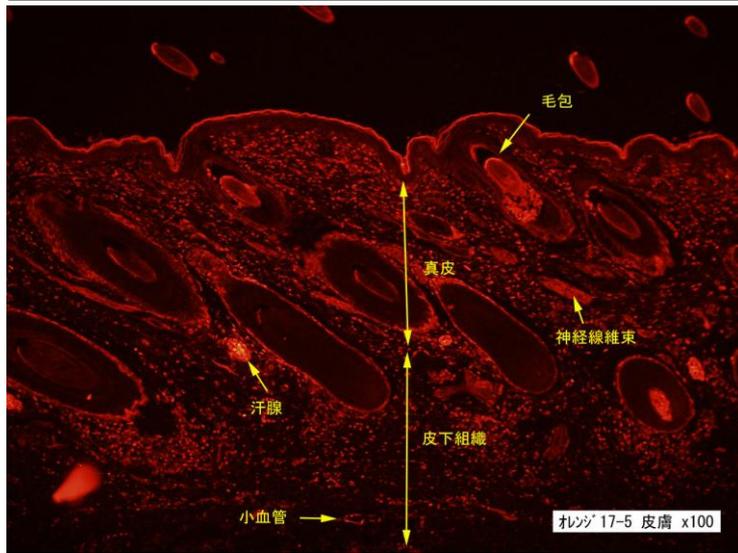
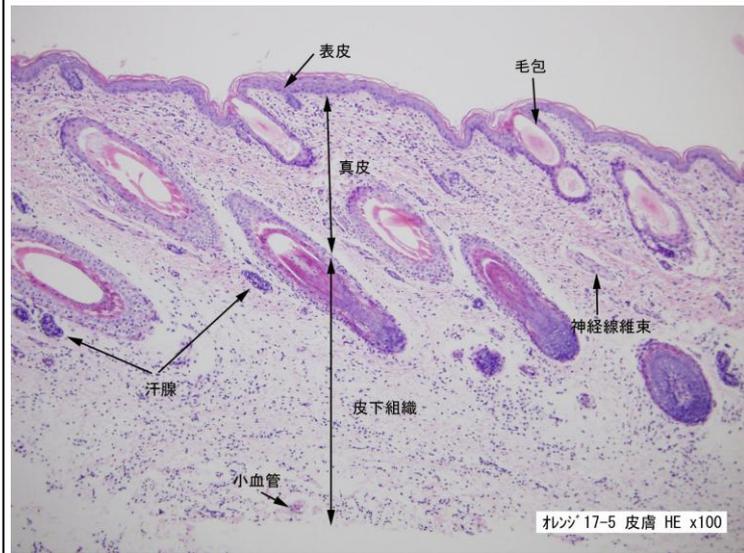
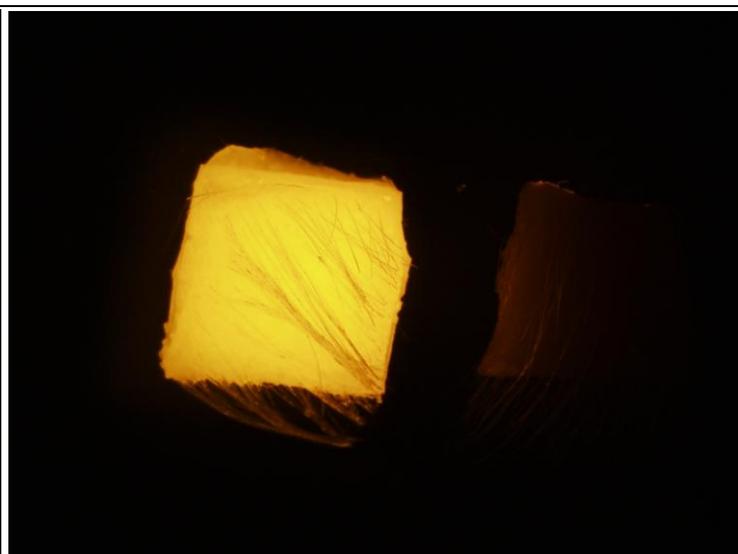
# huKOブタ臓器・組織の蛍光発現:肝臓



明視野像

蛍光発現像

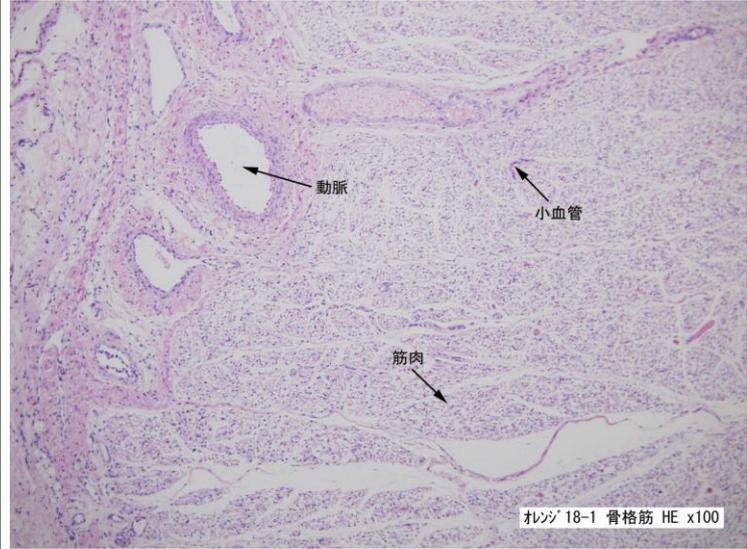
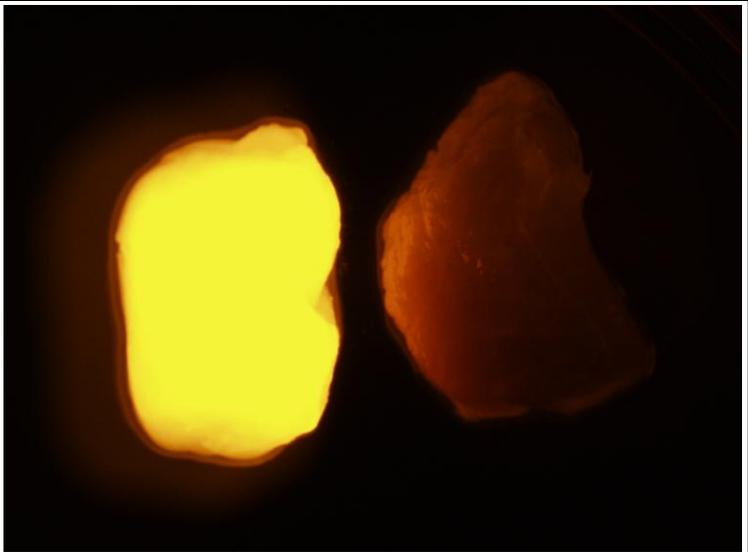
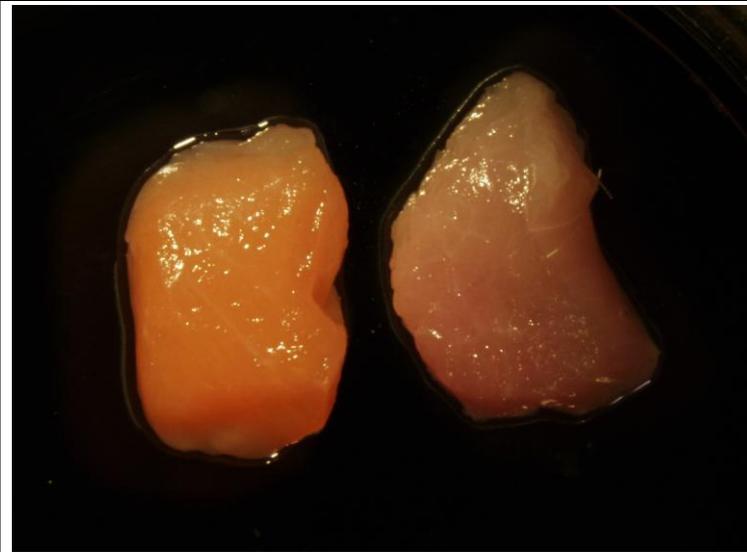
# クサビラオレンジブタ臓器・組織の蛍光発現:皮膚



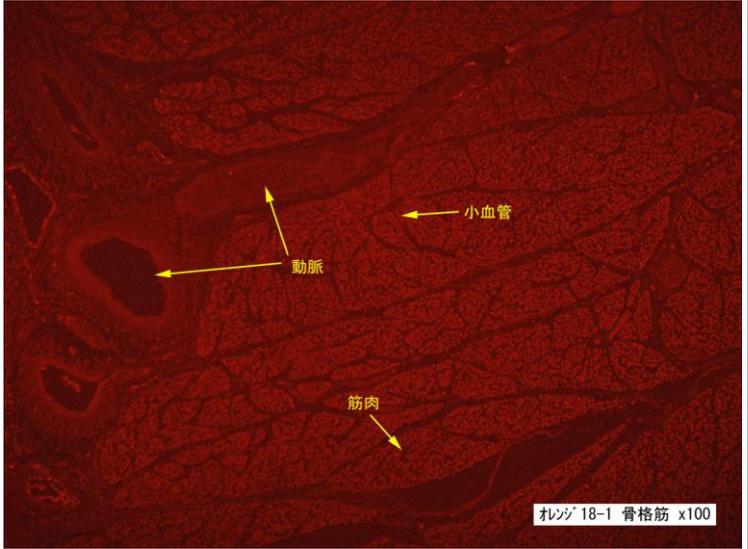
明視野像

蛍光発現像

# huKOブタ臓器・組織の蛍光発現:骨格筋

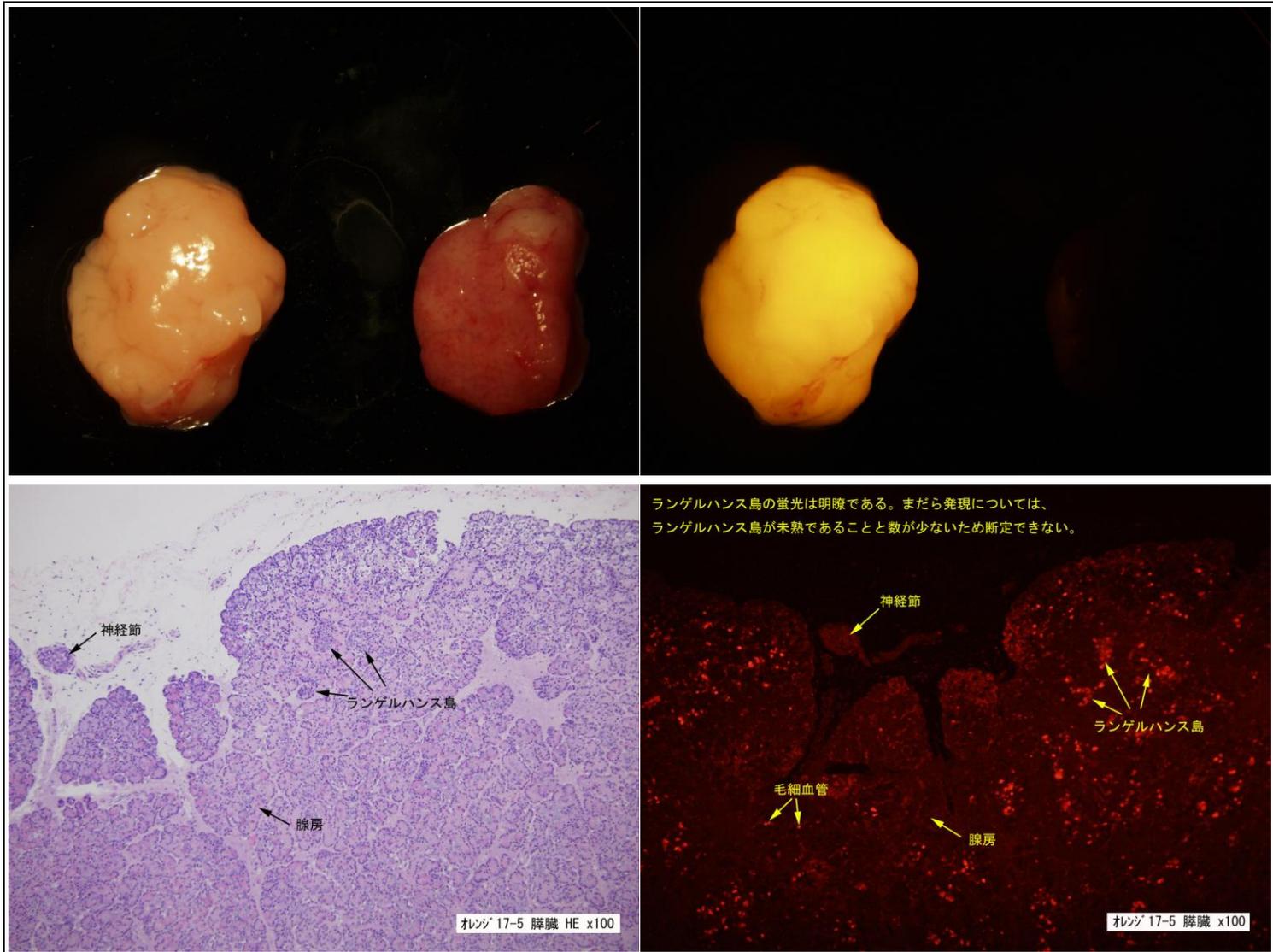


明視野像



蛍光発現像

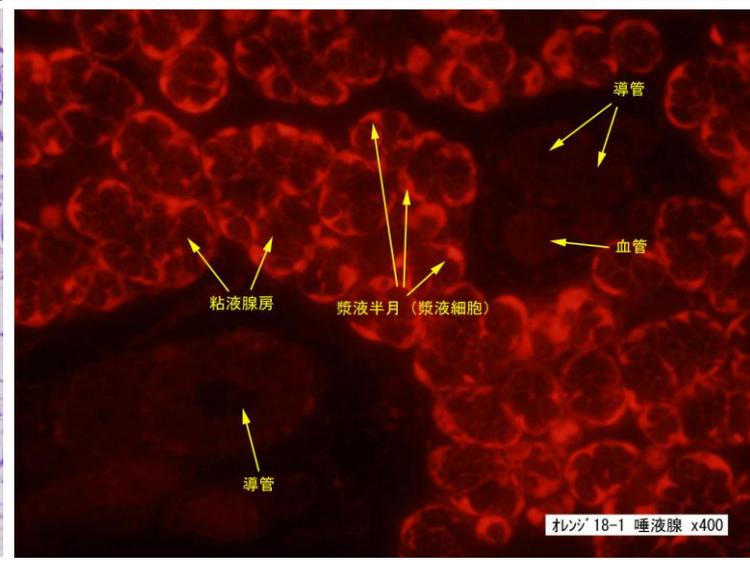
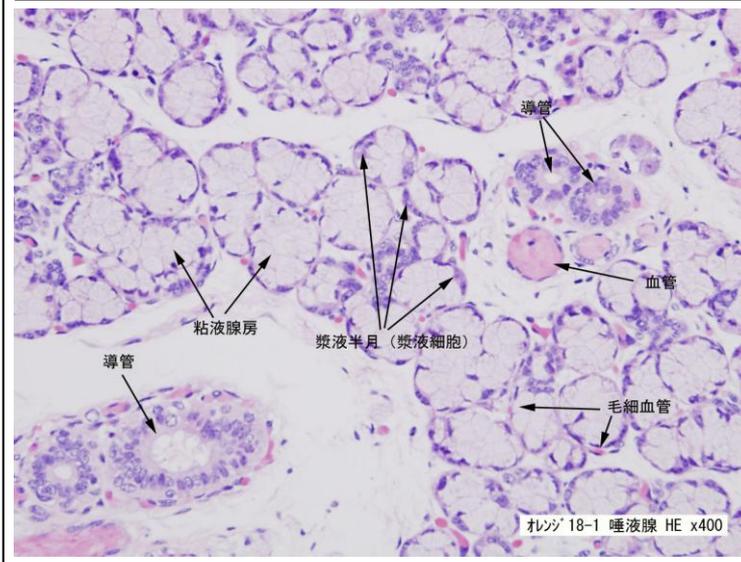
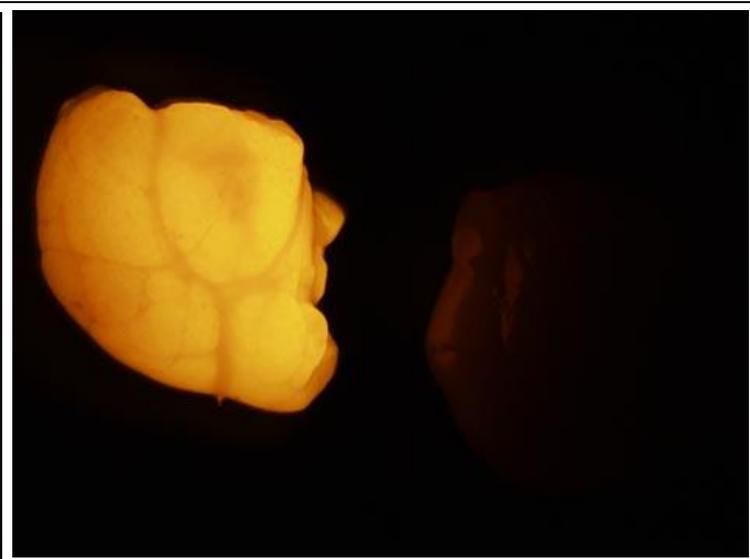
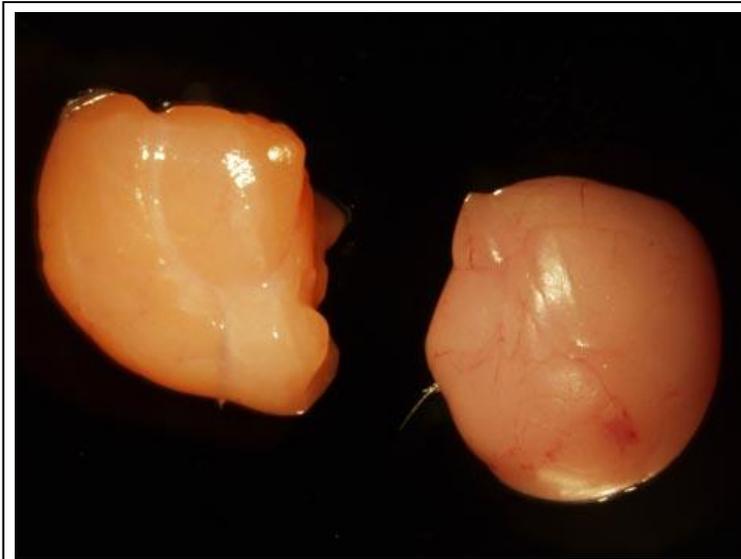
# huKOブタ臓器・組織の蛍光発現:膵臓



明視野像

蛍光発現像

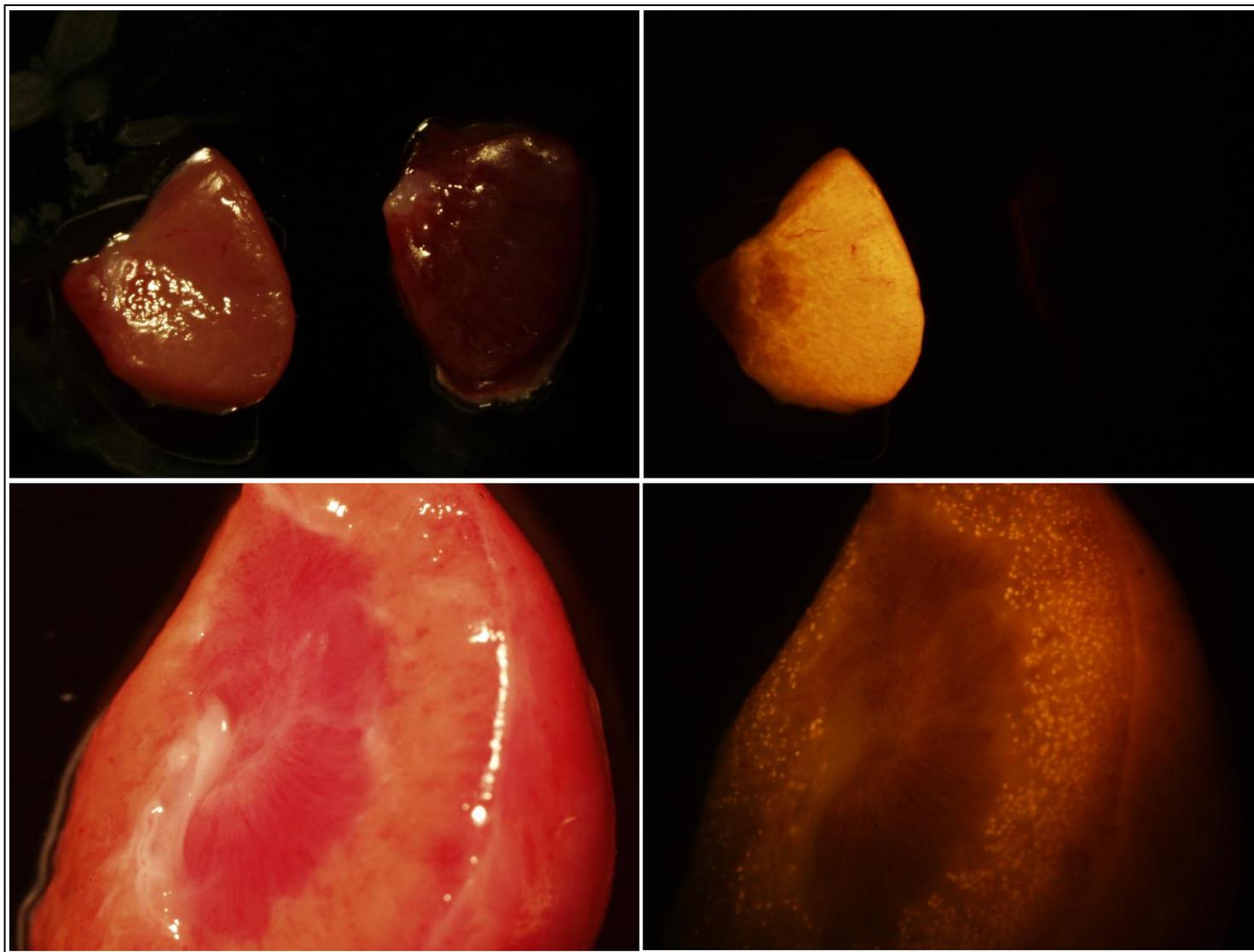
# huKOブタ臓器・組織の蛍光発現: 唾液腺



明視野像

蛍光発現像

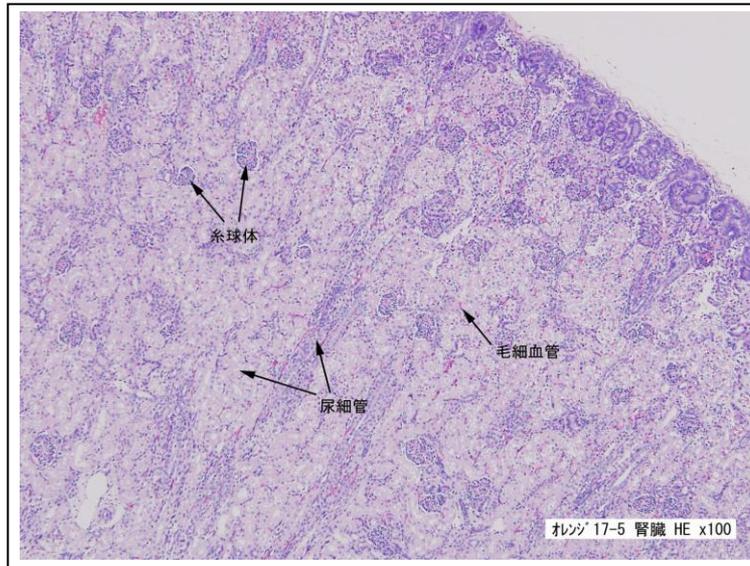
# クサビラオレンジブタ臓器・組織の蛍光発現:腎臓



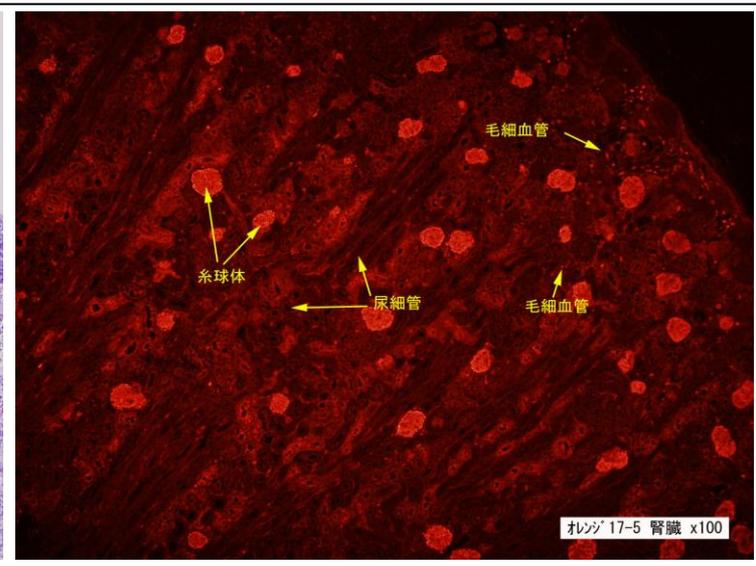
明視野像

蛍光発現像

# クサビラオレンジブタ臓器・組織の蛍光発現:腎臓

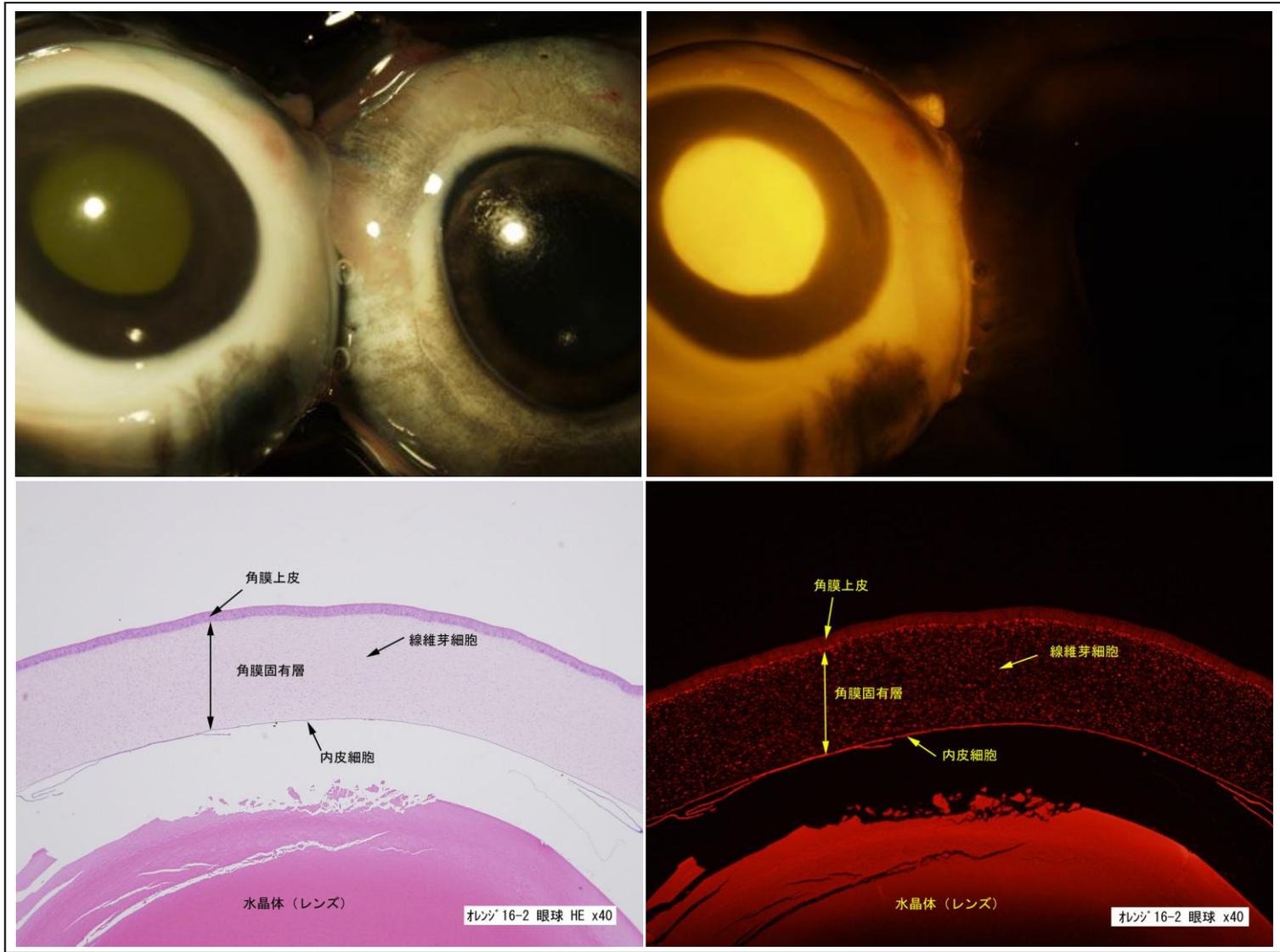


明視野像



蛍光発現像

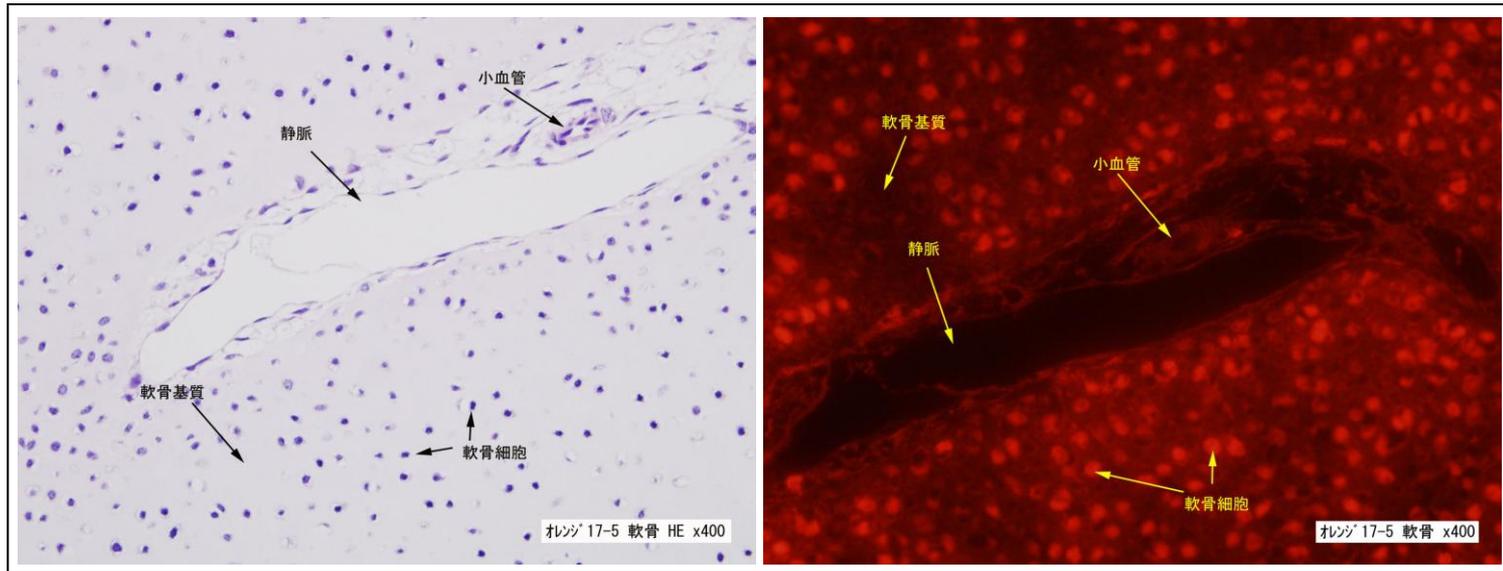
# クサビラオレンジブタ臓器・組織の蛍光発現:眼球



明視野像

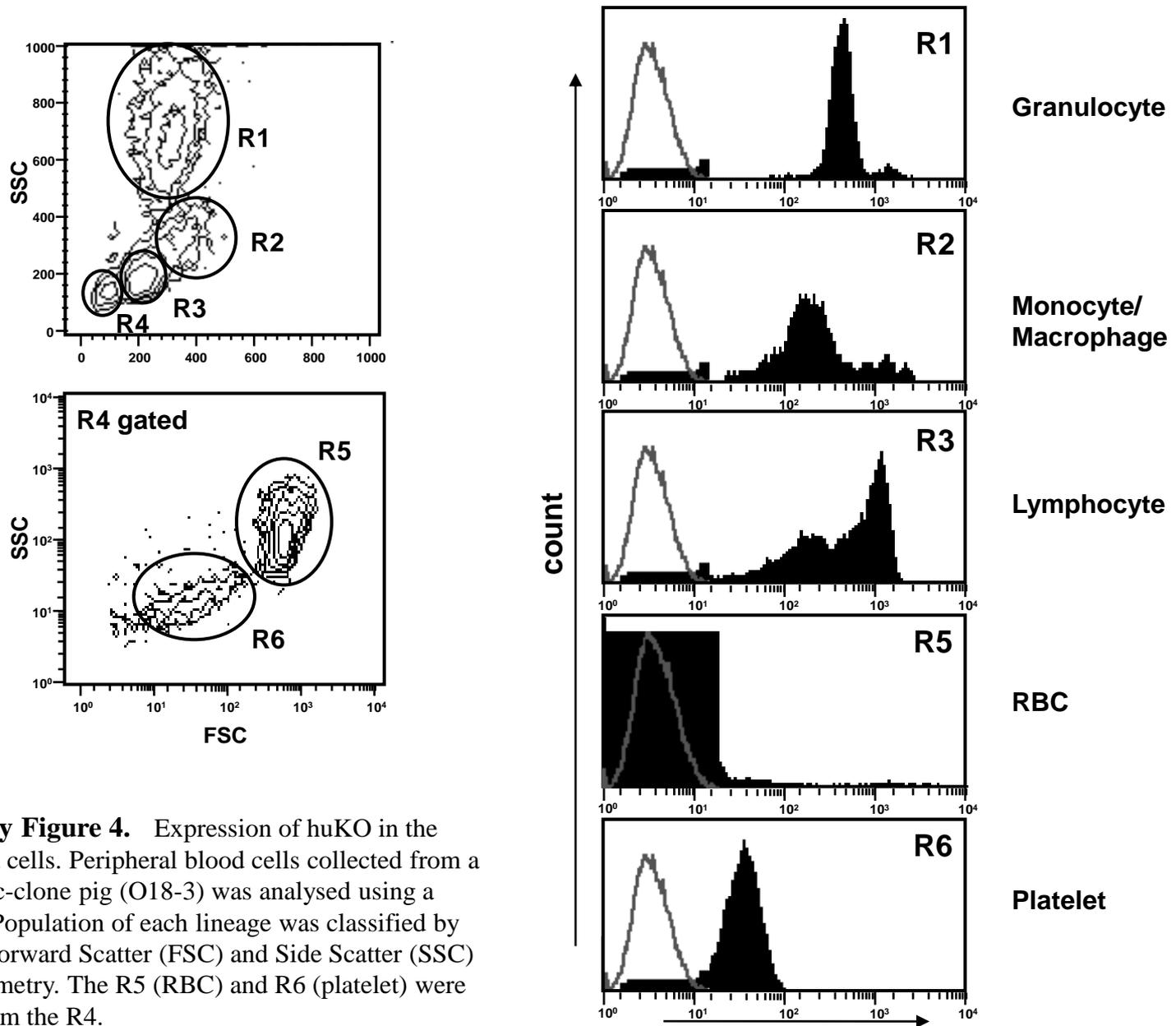
蛍光発現像

# クサビラオレンジブタ臓器・組織の蛍光発現:軟骨

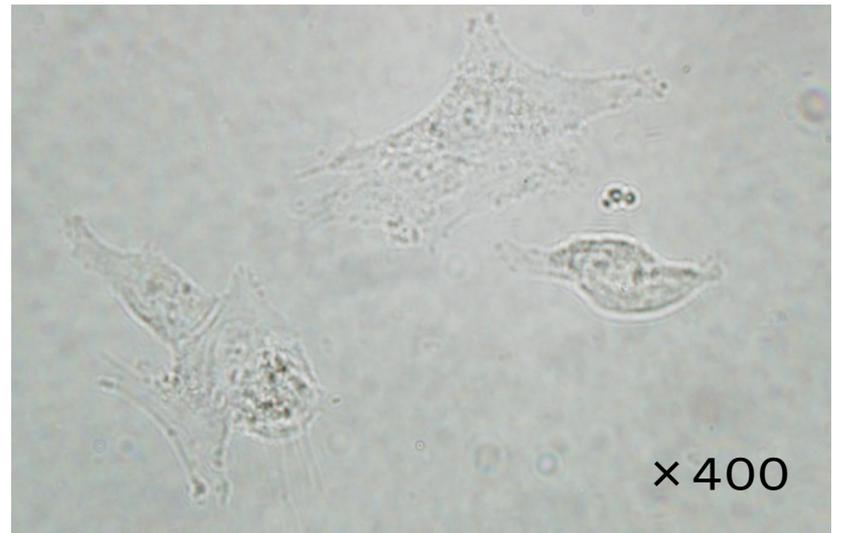
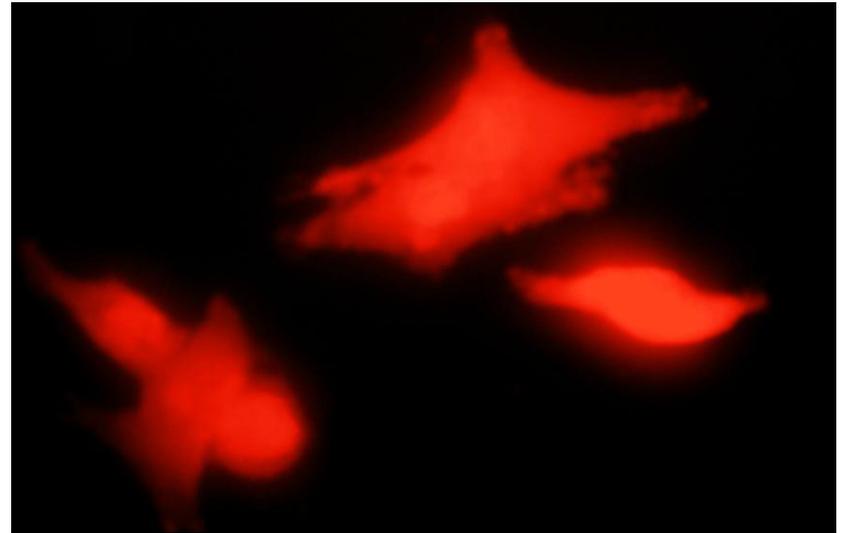
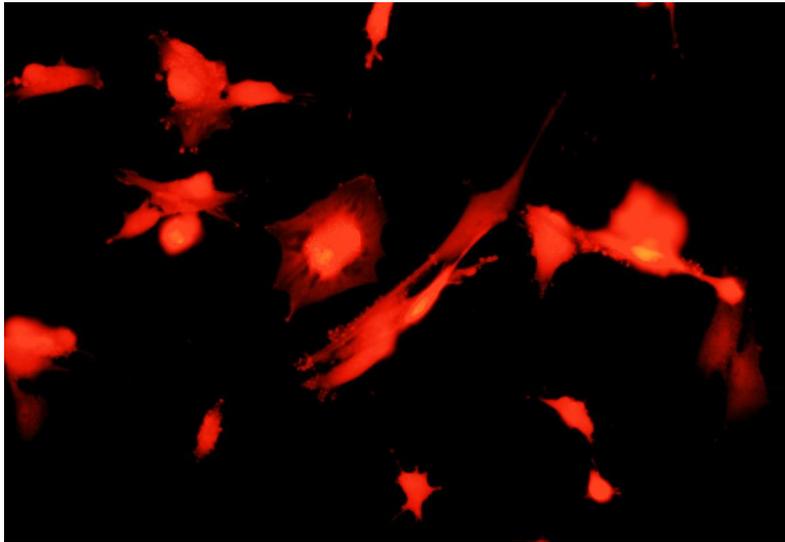


明視野像

蛍光発現像

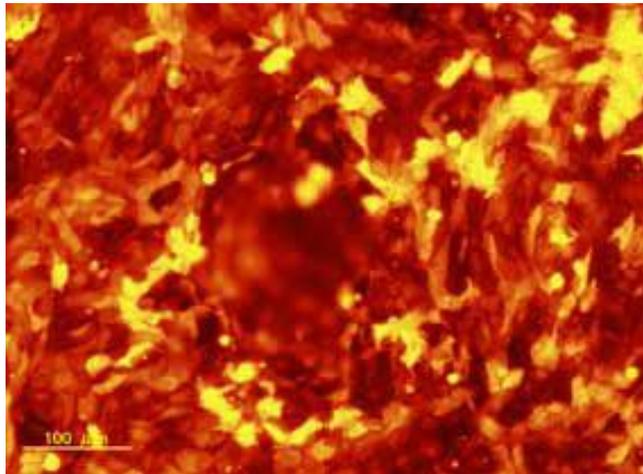
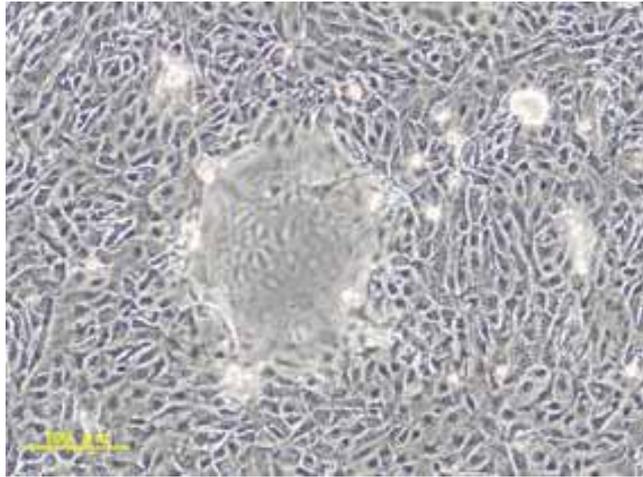


**Supplementary Figure 4.** Expression of huKO in the peripheral blood cells. Peripheral blood cells collected from a huKO transgenic-clone pig (O18-3) was analysed using a FACS-Calibur. Population of each lineage was classified by values of both Forward Scatter (FSC) and Side Scatter (SSC) of the flow cytometry. The R5 (RBC) and R6 (platelet) were further gated from the R4.

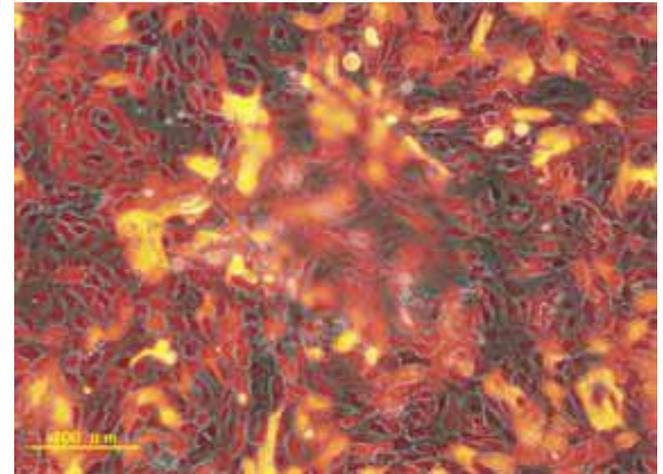


**huKO遺伝子導入クローンブタから樹立した骨髄間葉系幹細胞(MSC)**

(自治医科大学 小林英司先生より)

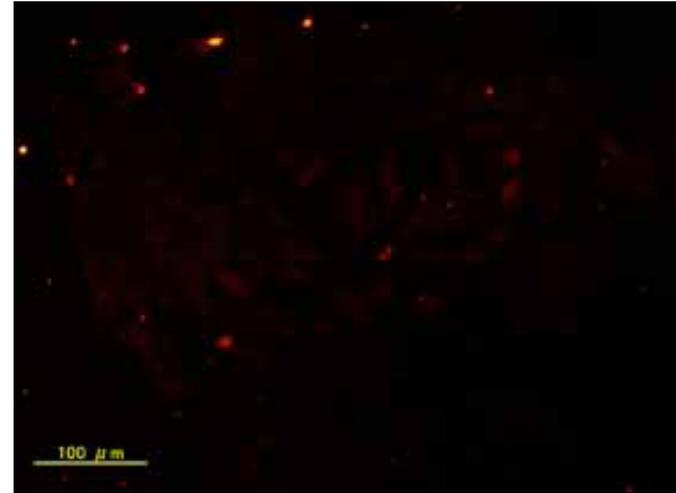
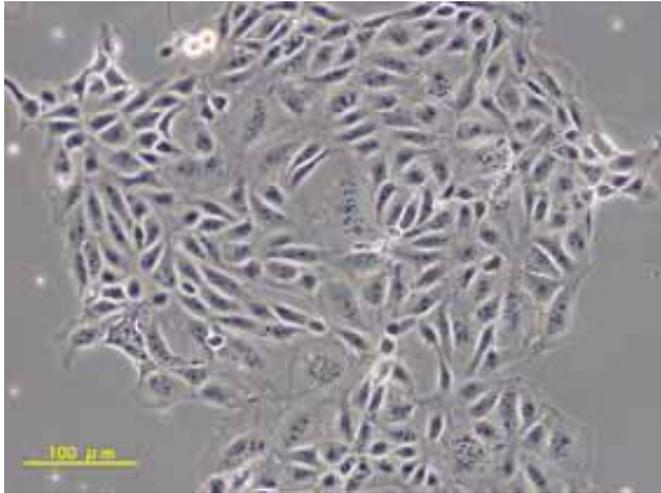


merge

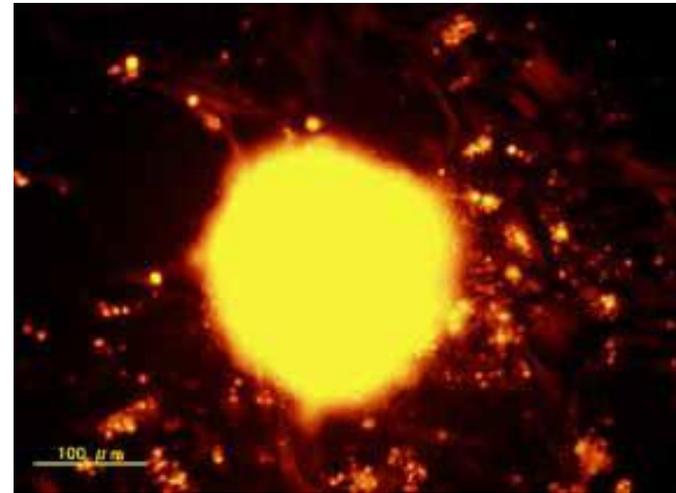
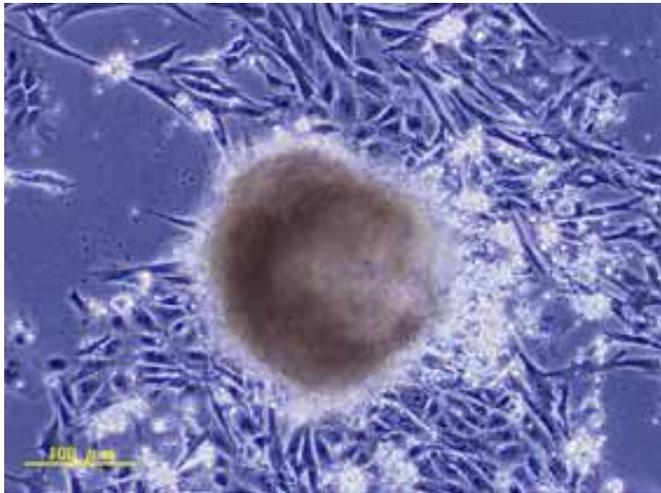


**huKO遺伝子導入クローンブタ唾液腺から樹立した内胚葉系幹細胞**  
(熊本大学 松本志郎先生より)

progenitor



islet



膵内分泌前駆細胞と分化誘導後のislet

(熊本大学 松本志郎先生より)

# Syngenicなドナー・レシピエント系

