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Preclinical Research

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ORIGINAL ARTICLE

SLUG is required for SOX9 stabilization and functions to promote cancer stem cells and metastasis in human lung carcinoma

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Cancer stem cells (CSCs) are a promising target for cancer therapy, particularly for metastatic lung cancers, but how CSCs are regulated is largely unknown. We identify two proteins, SLUG (encoded by *SNAI2* gene) and SOX9, which are associated with advanced stage lung cancers and are implicated in the regulation of CSCs. Inhibition of either SLUG or SOX9 sufficiently inhibits CSCs in human lung cancer cells and attenuates experimental lung metastasis in a xenograft mouse model. Correlation between SLUG and SOX9 levels was observed remarkably, we therefore sought to explore their mechanistic relationship and regulation. SLUG, beyond its known function as an epithelial–mesenchymal transition transcription factor, was found to regulate SOX9 by controlling its stability via a post-translational modification process. SLUG interacts directly with SOX9 and prevents it from ubiquitin-mediated proteasomal degradation. SLUG expression and binding are necessary for SOX9 promotion of lung CSCs and metastasis in a mouse model. Together, our findings provide a novel mechanistic insight into the regulation of CSCs via SLUG-SOX9 regulatory axis, which represents a potential novel target for CSC therapy that may overcome cancer chemoresistance and relapse.

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INTRODUCTION

Lung cancer is the leading cause of cancer death that kills more than one million people worldwide each year.[1] The poor survival rate of patients is largely attributed to diagnosis at late stages with local or advanced metastasis at distant organs.[2,3] Although recent chemotherapy and radiotherapy have improved palliation, the treatment outcomes remain poor, as metastasis is largely incurable. In the past decade, subpopulations of cancer stem cells (CSCs; also known as tumor-initiating cells) have been reported in many solid tumors including breast, prostate, colon and lung,[4–6] which appear to both initiate the bulk of tumors and drive their progression through continuous rounds of self-renewal.[4,7,8] CSCs can acquire apoptosis resistance and increased cell migratory and invasive properties, a prerequisite for tumor metastasis.[9,10] The presence of CSCs in primary tumors is strongly correlated with an increased incidence of metastasis and poor survival of patients,[11–13] suggesting them to be promising target for cancer therapy. Here, we compared lung CSCs with their non-CSC counterpart and investigated the regulatory mechanisms that determine their metastatic behavior.

As epithelial–mesenchymal transition (EMT), a trans-differentiation process by which cells undergo a morphological change into a more mesenchymal phenotype, is common occurrence in metastasis of lung and other tumors,[14,15] we profiled EMT and identified SLUG (encoded by *SNAI2* gene) as significantly upregulated in the tested lung CSCs. SLUG is a member of Snail family with a unique conserve motif near the zinc fingers that is absent in other members.[16] A high expression of *SNAI2* is found in highly invasive lung cancer cells and tumor specimens, and is associated with poor survival and cancer relapse.[17,18] We further observed here that SLUG is not required for EMT activation in lung cancer cells, leading us to the discovery of other pathways that may contribute to the aggressive phenotypes of lung CSCs.

CSCs and normal stem cells share many common characteristics, for example, self-renewal and differentiation. Correlations between the regulatory pathways critical for normal developmental process and tumor progression have long been hypothesized and are being recognized.[19–21] Sex-determining region Y-boxes (SOX) family is known to have a pivotal role in the regulation of embryonic development and its members have been used as pluripotent stem cell markers.[22] SOX9, in particular, is expressed in lung epithelium and mesenchyme, and is critical in tracheal differentiation and formation.[23] Upregulation of SOX9 has been reported in lung adenocarcinoma, supporting its clinical significance in lung cancer.[24] We demonstrate here the high-level SOX9 in correlation with high-level SLUG in lung CSCs and advanced stage lung cancers. Thus, we further investigated: (a) the roles of SLUG and SOX9 in lung CSCs and metastasis; (b) the SLUG and SOX9 relationship; and (c) their regulatory mechanisms. Our findings could be important in understanding CSCs and lung

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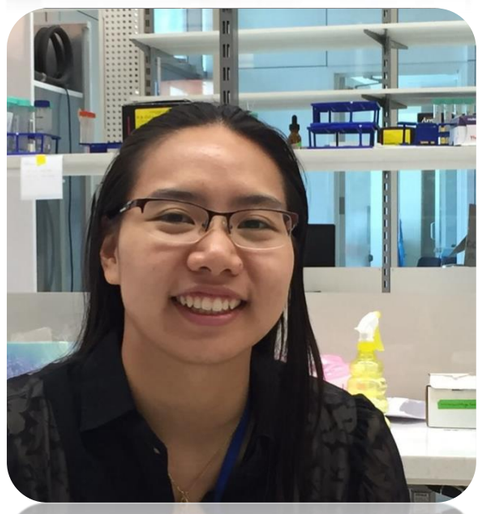

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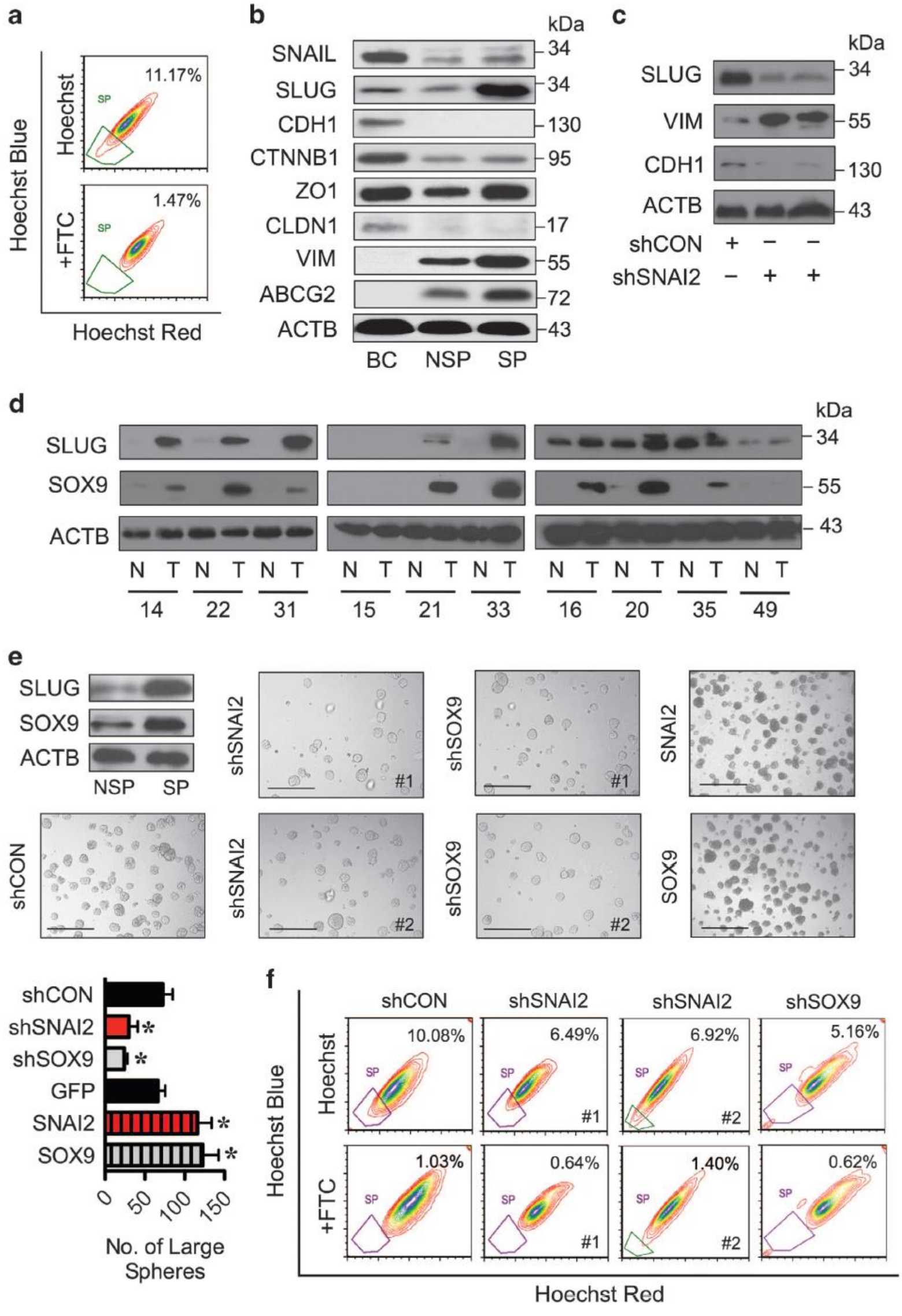

Figure 1. Lung CSCs and clinical lung carcinoma exhibit high levels of SLUG and SOX9.

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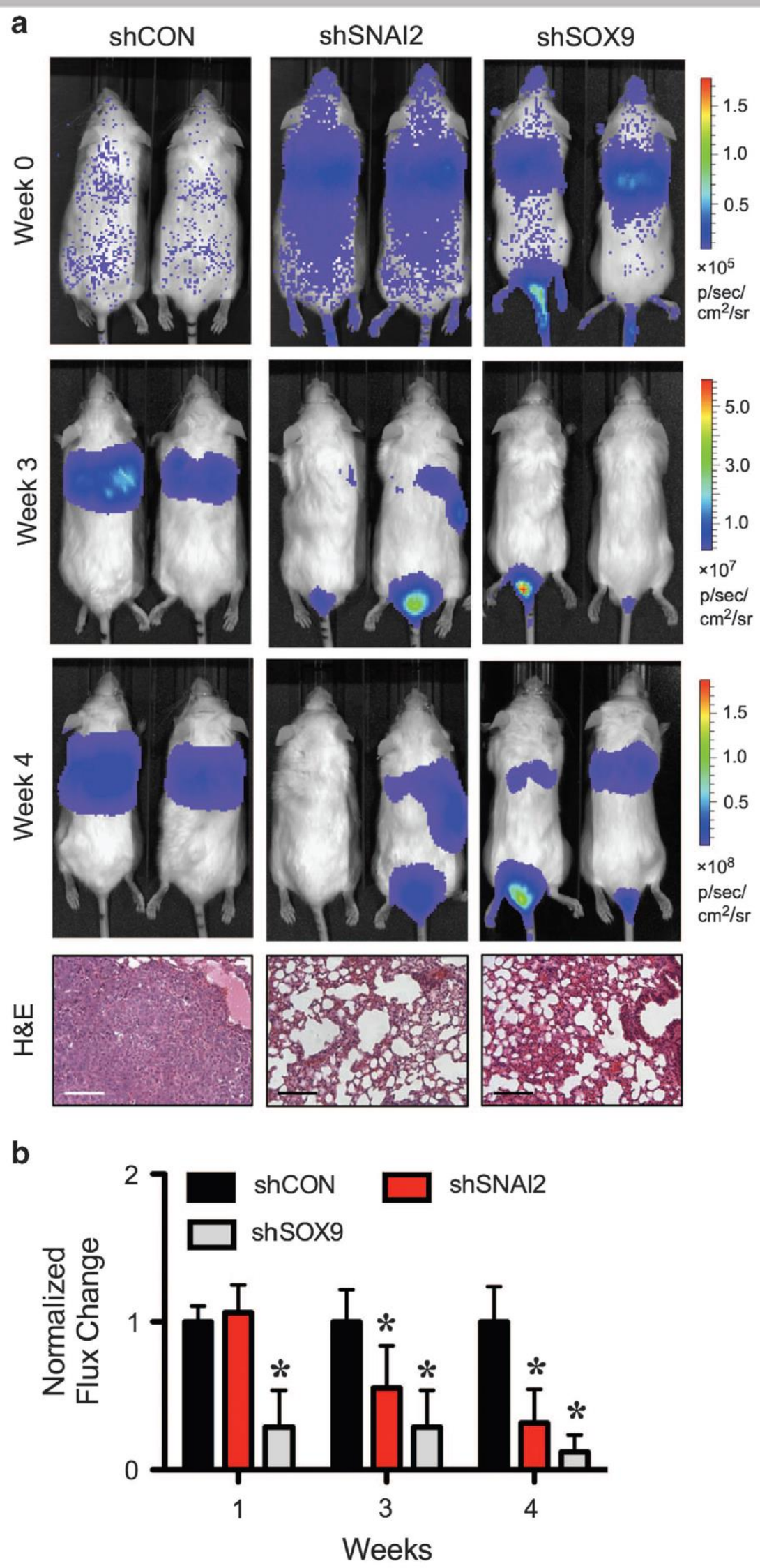

Figure 2. Inhibition of SLUG and SOX9 suppresses experimental lung cancer metastasis in vivo. LUC2-labeled shSNAI2, shSOX9 or shCON H460 cells were injected into NSG mice via tail vein at the dose of 1×10^6 cells/mouse.

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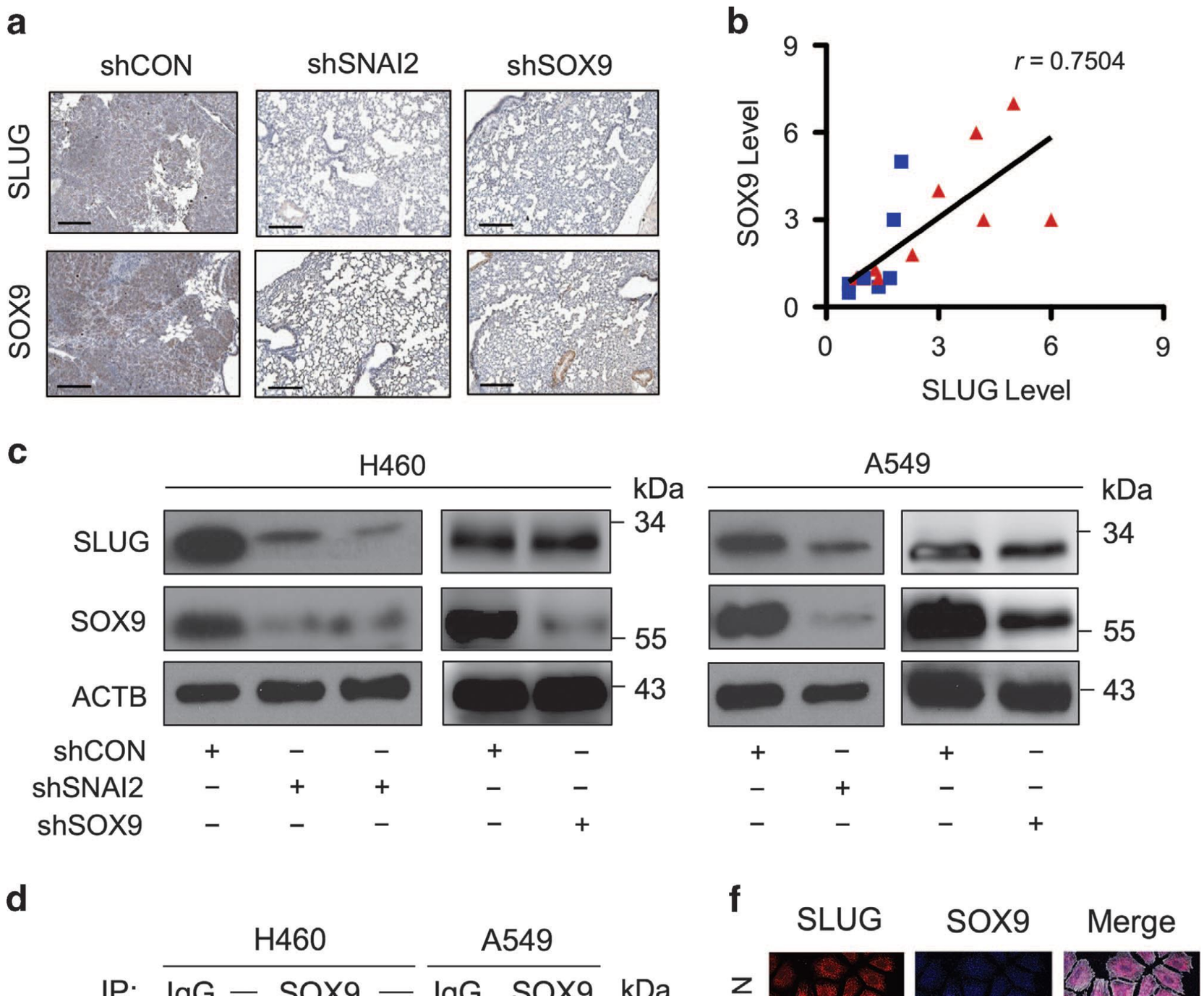

Figure 3. SLUG regulates of SOX9 in NSCLC cells.

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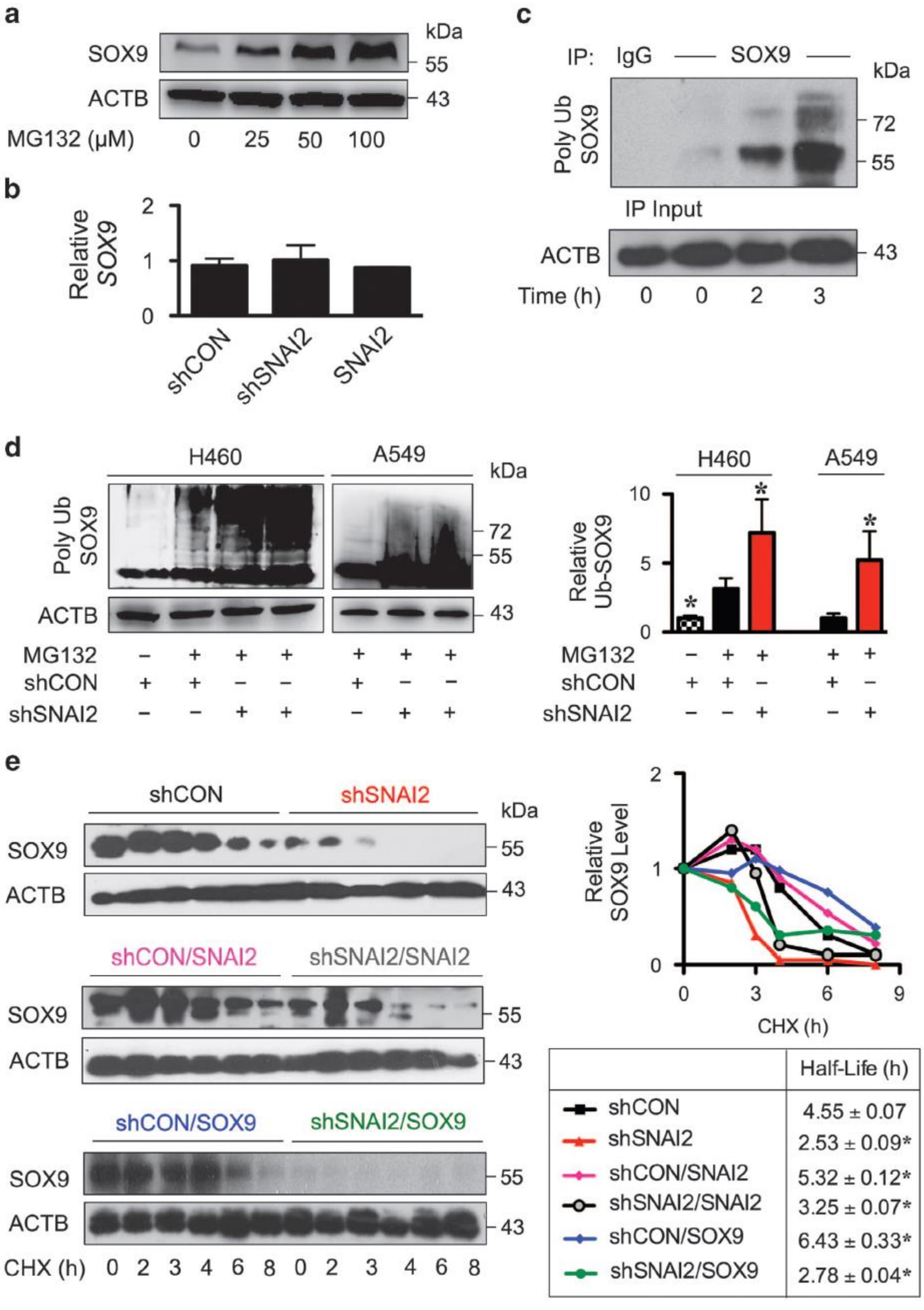

Figure 4. SLUG inhibits SOX9 ubiquitination and prolongs its stability.

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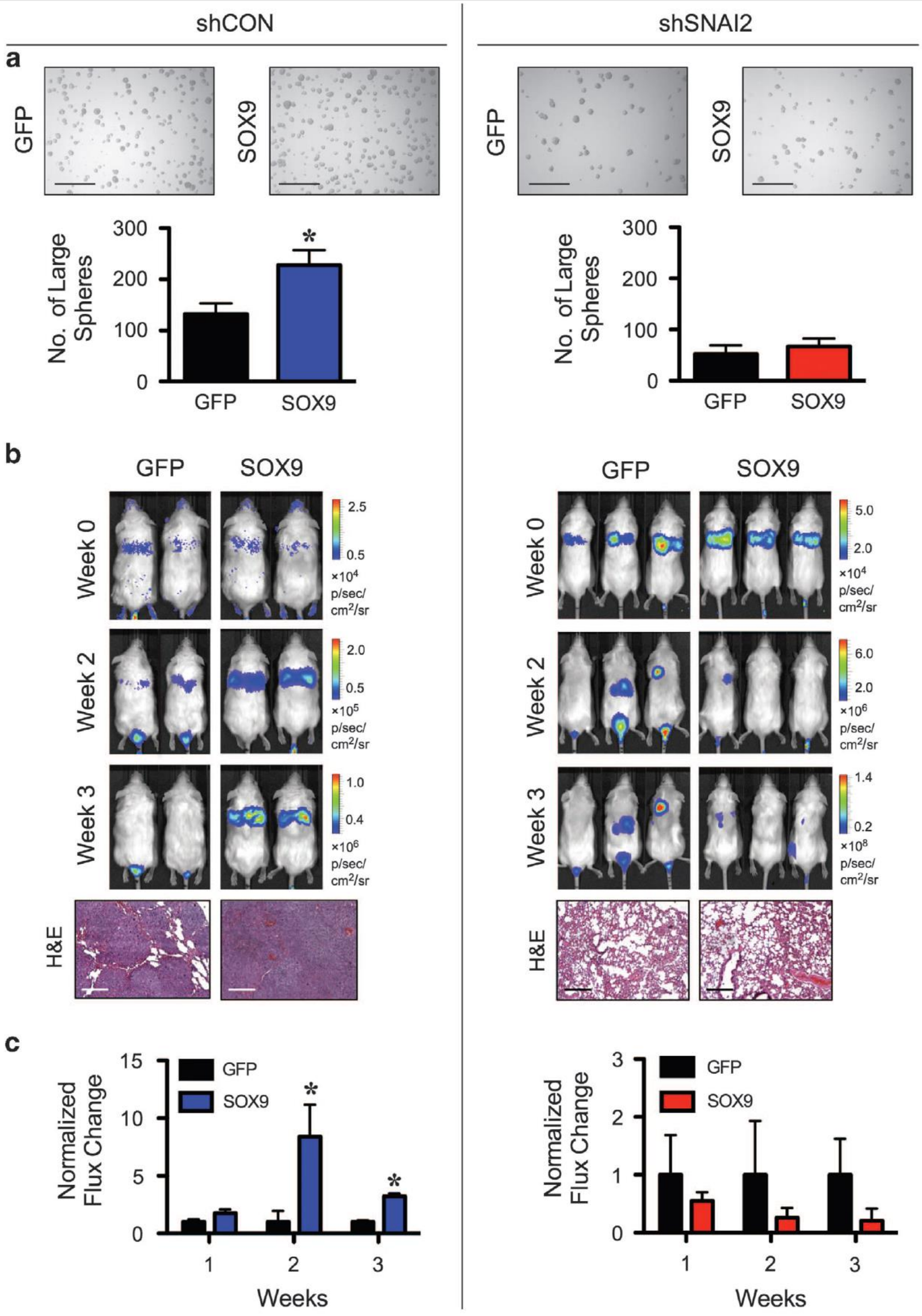

Figure 5. SLUG is required in SOX9-mediated lung CSC in vitro and metastasis in vivo.

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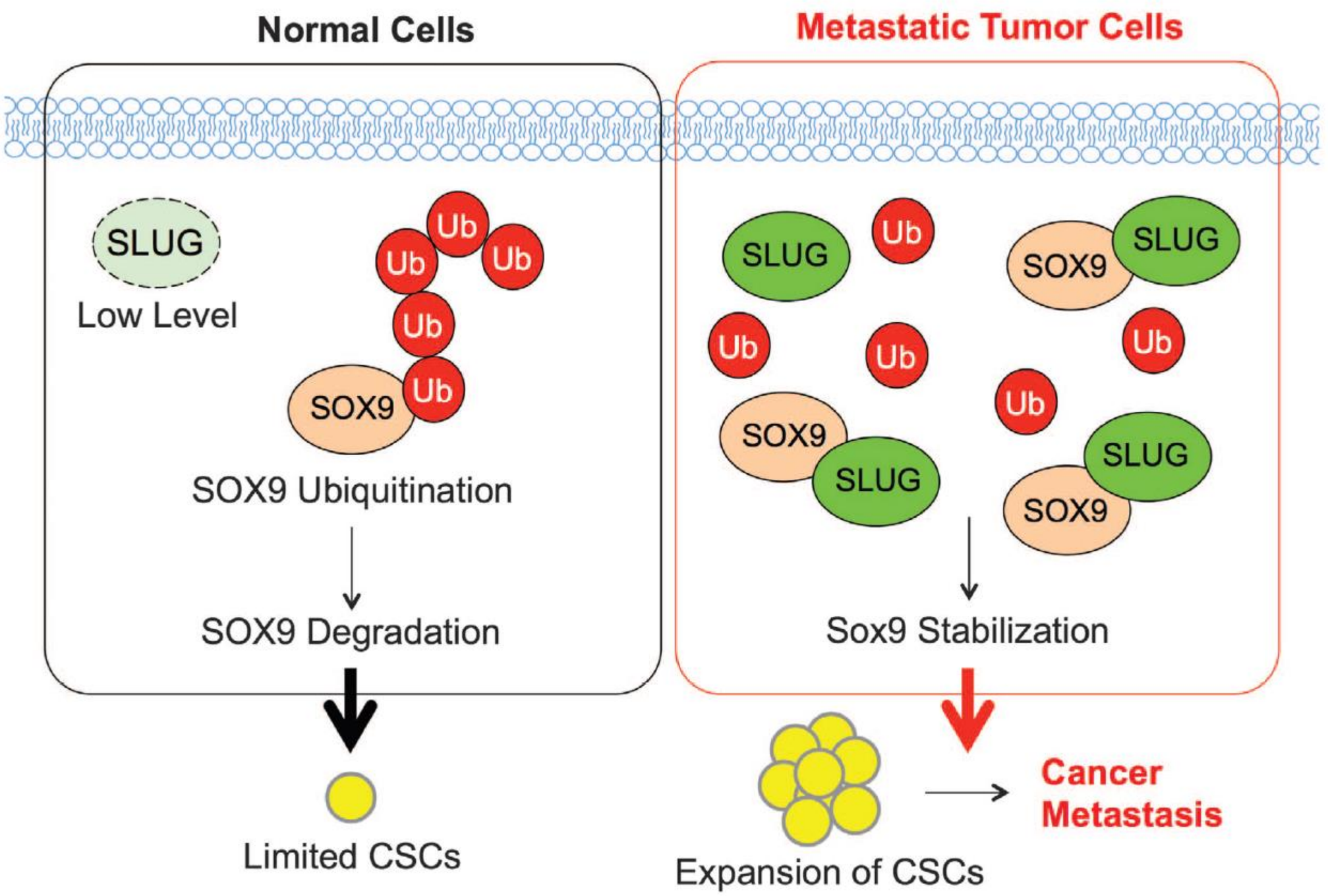

Figure 6. A schematic working model for the function of SLUG-SOX9 axis in CSC and metastasis regulation. In metastatic tumor cells, increased SLUG expression stabilizes SOX9 through their binding interaction, which inhibits SOX9 ubiquitination and proteasomal degradation. SOX9 stabilization promotes the expansion of CSCs and subsequent cancer metastasis.